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FOMES ANNOSUS

Proceedings of the Fourth International Conference on Fomes Annosus

Athens, Georgia
September 17–22, 1973

International Union of Forest Research Organizations
Section 24: Forest Protection

Forest Service
United States Department of Agriculture, Washington, D.C.
1974

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Proceedings of the Fourth International Conference on Fomes Annosus

Athens, Georgia
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Edited by E. G. Kuhlman

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PHOTOGRAPH

Front row (left to right): Kuhlman, Dimitri, Morrison, Collins, Roll-Hansen, Pratt, Lundeberg; 2nd row: Azevedo, Sylvestre, Redfern, Lukomski, Klingström, Wallis; 3rd row: Driver, Greig, Froelich, McAree, Koenigs; 4th row: Aufsess, Alcubilla, Yde-Andersen, Skelly, Rishbeth; 5th row: Parmeter, Shain, Courtois, Kallio, Alexander, Wolfe; 6th row: Stambaugh, Cowling, Witcher, Padgett, Ross, Lane; and 7th row: Russell Kauffman, Fleming, Johansson, Srago, Myren, Krebill, Cobb.

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INTRODUCTION

The Fourth International Conference on Fomes annosus was held September 17-22, 1973, at Athens and Bainbridge, Georgia, with the U. S. Forest Service, the University of Georgia, and International Paper Company as hosts. More than 50 scientists from 14 countries were present. George Kuhlman and A. Yde-Andersen served as Conference Chairman and IUFRO Working Group Chairman, respectively. Thirty papers were presented in eight lively discussion sessions with the following moderators: John Rishbeth, A. Yde-Andersen, Richard Parmeter, Louis Shain, Eldon Ross, Finn Roll-Hansen, Charles Driver, and Charles Hodges. The papers and some highlights from the discussion are presented in these proceedings.

At an evening banquet Bruce J. Zobel, forest geneticist, North Carolina State University, presented an illustrated lecture on his program for improving the southern pines.

Tom Miller and Wiley Garrett led tours of the Forestry Sciences Laboratory and the Plant Pathology Department in Athens. Near Bainbridge at the Southlands Experimental Forest of International Paper Company, Ron Froelich, Eldon Ross, and Charles Driver showed their field studies on Fomes annosus. John Stone, Roger Blair, James Buckner, and L. V. Collicott of the Southlands staff discussed aspects of their research program. Roger M. Copeland represented the Georgia Center staff in hosting the portion of the conference held in Athens.

FOMES ANNOSUS AS A FACTOR IN THE MANAGEMENT OF RECREATIONAL FORESTS

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INTRODUCTION

The potential of Fomes annosus (Fr.) Cooke to cause mortality and volume loss in timber stands is well documented. The impact of F. annosus on stands of special historic, esthetic, or recreational value has received little attention. With increasing emphasis on the management of forest stands for recreational use, damage by F. annosus may become acute in many areas. Yosemite Valley is a good example.

Yosemite Valley, at the time it became a public trust in 1864, was mainly covered with meadows and open, oak woodlands. Conifers were scattered throughout at low densities. As a result of fire control, overgrazing and meadow drainage, dense stands of ponderosa pine (Pinus ponderosa Dougl.) with scattered incense-cedar (Libocedrus decurrens Torr.) developed over much of the valley floor, gradually shading out many of the oaks (4). Today these overstocked, nearly even-aged stands are about 80-100 years old. The mean diameter of 1459 measured pines was 27.2 inches at breast height, but many dominant trees run 3-4 feet or more in diameter. Most of the camping, cabin and concessionaire developments are located in these pine-cedar stands.

Windthrow and killing of trees by bark beetles have been continuous problems in Yosemite Valley. In 1966, F. annosus was identified from

the rotted roots of a windthrown pine. Subsequent identification of F. annosus in several areas of chronic bark-beetle activity led the Park Superintendent to request an evaluation of the disease problem. The evaluation is being conducted by the California Region of the Forest Service in cooperation with the University of California. Early results point to serious problems in the management of recreational forests and to important deficiencies in our knowledge of the behavior and control of F. annosus.

INCIDENCE AND SPREAD OF F. ANNOSUS IN YOSEMITE VALLEY

All developed sites (campgrounds, cabin sites, and commercial areas) in Yosemite Valley were systematically examined. Wherever two or more coniferous stumps or one stump and a dead or dying tree were found, root isolations were made on a selective medium (7). Sporophores provided positive identification without isolation. One hundred F. annosus centers were confirmed in the 24 developed sites that were examined. Although undeveloped sites were not systematically surveyed, 44 additional centers were found by random walks, aerial photographs, or observations from high cliffs surrounding the valley. Some centers were sufficiently large or boundaries so indistinct that they may have represented two or more coalescing centers. Conversely, some centers were so close that they may have represented peculiarities in the pattern of spread from a single center. Despite these uncertainties with respect to exact numbers of centers, the results indicate a widespread and serious incidence of F. annosus in Yosemite Valley.

During the summer of 1971, 88 disease centers and surrounding live trees were carefully mapped and the diameter and condition of each tree or stump was recorded. Mapped centers totaled 5.9 acres. Center sizes ranged from 0.0+ - 0.05 acres (54 centers), 0.06 - 0.10 (19 centers), 0.11 - 0.20 (10 centers), to 0.21-0.58 acres (5 centers). Dead trees and stumps included 506 ponderosa pines (mean diameter 23.5 in.), 99 incense-cedars (mean diameter 13.7 in.), and 8 white firs (mean diameter 18.0 in.). Some of these trees, especially those from which the centers originated, may have been killed by agents other than F. annosus. However, examination of old Park Service maps indicated that some past windthrows or stumps cut flush with the ground and buried were not identified. While numbers therefore were not exact, the evidence indicated that several hundred trees have been killed as a result of F. annosus activity.

Revisits to mapped centers in 1972 showed that 65 additional trees had died. Most centers, including the largest ones (0.20-0.58 acres), had one or more trees dead or showing symptoms of decline. These observations plus the presence of fresh sporophores or viable mycelium indicated that the fungus was active in all centers. We have found no evidence that any centers have been "arrested" or that centers in dense stands will not continue to enlarge until natural barriers or elimination of hosts preclude further spread.

Limited observations provided a preliminary estimation of the rate of spread of F. annosus in Yosemite Valley centers. Three centers (one was among the largest encountered) occurred in a campground that had

been mapped in detail in 1937, with every tree and stump accurately plotted. Each of the three centers had only one or a few stumps in 1937. Assuming that the stumps were infected at that time, the rate of spread since 1937 necessary to account for the present size of centers was 3-5 feet per year. This rate is within the range of rates discussed by Hodges (5). At this rate, the centers in Yosemite Valley varied from 1 to slightly more than 35 years old.

The distance as well as the rate that F. annosus can spread from one large tree to another is critical in assessing future impact. Examination of mapped centers indicated that spread between large trees 30-40 feet apart was common. Numerous examples of spread at greater distances, several in the 60-90 foot range, were encountered. Because extensive excavations could not be made in the Park, we do not know whether these greater distances represent root-to-root spread or spread by some other mechanism. Since ponderosa pine root systems can extend radially for 100 feet or more (2), root spread over considerable distances between large trees may be possible.

The origin of the F. annosus problem in Yosemite Valley is unknown. Since early descriptions of vegetation suggest that pines were scattered, it is unlikely that appreciable infestation occurred before present stands were established. Some cutting has been done throughout the history of the Valley, but cutting, campground establishment, ditching, and other disruptions of pine stands were intensified in the 1930's, when abundant labor was available. Tree cutting for insect control and hazard reduction has continued to the present. Evidence suggests that most large centers were established in the 1930's and that the initiation of new centers has continued to the present.

Because stands in developed sites were predominantly pine and incense cedar, most F. annosus centers were nearly denuded. Of 2651 live trees on or surrounding mapped centers, 2526 were conifers, 89 were black oaks (Quercus kelloggii Neub.), and the remaining 36 were miscellaneous hardwoods, 25 of which were less than 5 in. in diameter. Unfortunately we do not have adequate information on the susceptibility of the various hardwoods to F. annosus in Yosemite Valley. Such information is needed to plan for reforestation of denuded sites.

HAZARD

Root-diseased trees can be hazardous to people, buildings, and cars. Paine (8, 9) and Wagener (11) have documented this hazard in California. In Yosemite Valley, two types of hazard were associated with F. annosus: (i) failure of trees with decayed roots, and (ii) breakage or windthrow of trees exposed by root-disease openings in the forest canopy. Four deaths have occurred in Yosemite Valley in the past 13 years, each involving trees root-rotted by F. annosus or trees in stands opened up by F. annosus. Many cases of damage to cars or buildings have been recorded, and potentially damaging tree failures have been frequent.

Data on the contribution of F. annosus to tree hazard were obtained in 1971. On December 7, a high wind caused the failure of 30 live

conifers. Of 10 ponderosa pines (24-36 in. in diameter), 16 incense-cedars (10-45 in. in diameter), and 4 white firs (11-36 in. in diameter) that failed, 6 pines and 10 cedars were windthrown because of extensive root decay by F. annosus. One pine that broke at a stem gall caused by Peridermium harknessii Moore and one incense-cedar that broke in midbole had been exposed by stand openings resulting from F. annosus. Thus 18 failures were caused by or associated with F. annosus. One cabin, one lodge, seven tent frames and a number of electric lines were damaged. Of the remaining 13 trees, 6 had root decay caused by Armillaria mellea (Fr.) Quel., 4 were knocked over by a decayed oak that failed, and one broke at a P. harknessii gall. These observations illustrate the role of diseases in general and of F. annosus in particular in creating tree hazards.

Some trees could be recognized as hazardous before failure. Pines with F. annosus often showed thin crowns with slight to marked chlorosis. Resinosis in one or more large roots near the root collar was common. Incense-cedars also showed crown thinning with variable degrees of chlorosis, and one or more roots generally showed decay or sapwood discoloration similar to heartwood in appearance. Unfortunately, some pines and cedars showed no external indication of F. annosus prior to failure. Thus external symptoms could not be relied upon for recognition of some hazardous trees.

MANAGEMENT PROBLEMS

Fomes annosus in recreational forests poses several difficult problems for land managers, especially where part of the recreational value involves large trees. Root disease in such trees creates serious hazard, alters esthetic or recreational values, and complicates planning for future development and use. In the case of Yosemite Valley, available evidence suggests that the uncontrolled spread of existing centers will ultimately eliminate much of the pine forests in which camp and cabin facilities are now situated.

The lack of information on the behavior of F. annosus in stands of large trees, especially pines and cedars, makes consideration of possible controls difficult. Clear-cutting around centers or trenching to sever roots requires knowledge of the distance the fungus can spread undetected from definable centers. In dealing with a tree species that can have roots extending over 100 feet, present clearing recommendations (3) for other forest types may be inadequate. In addition, clearing of centers in stands of large trees may lead to serious windthrow hazards at exposed stand margins. Fumigation (6) might be feasible in high-value stands, but the size of roots on large trees suggests that fumigant penetration would be inadequate without root removal. At our present state of knowledge, no satisfactory solutions to these problems are apparent; nor is there evidence that natural factors will limit losses.

Trees of all ages have been killed, and there is no indication that damage will diminish as stands grow older. Spread and mortality have continued in the oldest, largest centers. Because of the difficulties and uncertainties in applying known controls and because no natural restrictions of center spread in dense stands have been observed, the

problem may become even more acute in Yosemite Valley, despite recent initiation of stump treatment. Unfortunately, Yosemite Valley may be only a single example of a much broader problem.

It is important to note that the problem in Yosemite Valley was largely unrecognized until a systematic survey was made. Fomes annosus is widespread in California (1) and has been reported from numerous recreational sites (M. Srago, unpublished Forest Service detection reports). Tegethoff (10) reported widespread F. annosus in the Rocky Mountains, including occurrences in ski and wintersport developments. We have observed numerous tree failures associated with F. annosus on high-use recreational and park sites. These observations and reports suggest that a systematic examination of such sites throughout the West likely will show serious F. annosus problems in some of the most valuable forest stands in the United States.

ACKNOWLEDGEMENT

We wish to acknowledge the special contributions of F. W. Cobb, Jr. and N. J. MacGregor, who participated in the planning of studies and in the preparation of this paper.

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ROOT PATHOGENS AS AGENTS PREDISPOSING PONDEROSA PINE AND WHITE FIR TO BARK BEETLES [1]

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INTRODUCTION

Studies have shown that bark beetles breed mainly in trees that are physiologically subnormal (4). Thus, any factor that adversely affects the health of trees may predispose them to bark beetles. Abiotic factors such as draught, flooding, fire, and lightning have long been implicated as agents of predisposition. More recently, air pollution injury has been shown to predispose trees to bark beetle infestations (6, 7). Biotic agents such as needle casts (2), dwarf mistletoe (2), true mistletoe (1), and root pathogens (6) have also been associated with beetle infestations but there are few reports (3, 6) that present definitive evidence on the extent and potential importance of such associations. Consequently, diseases have been largely discounted in studies of population dynamics or control of bark beetles.

The studies reported here were initiated in 1965 to obtain an initial estimate of the importance of root pathogens in predisposing

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[1] Study supported in part by NSF Grant No. GB-7363.

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ponderosa pine (Pinus ponderosa Doug.) and white fir [Abies concolor (Gord. and Glen.) Lindl.] to bark beetles (Coleoptera:Scolytidae).

METHODS

The association between root disease and bark beetle infestations in ponderosa pine was studied in four areas: (i) Georgetown Divide, El Dorado County, (ii) Boggs Mountain State Forest, Lake County, (iii) McCloud Flats, Siskiyou County, and (iv) Plumas National Forest, Plumas County. Similar studies were initiated in white fir only on the Georgetown Divide.

Moderate to high mortality of both ponderosa pine and white fir existed on the Georgetown Divide in the central Sierra Nevada. There were no major outbreaks of beetles, however, and the condition was considered chronic. Boggs Mountain, in the California Coastal Mountain Range, had moderate mortality in ponderosa pine; again, the condition was considered chronic. McCloud Flats, in the southern Cascade Mountains, had areas of high ponderosa pine mortality, and at the time of investigation, bark beetles were in an outbreak stage. The area studied on the Plumas National Forest (northern Sierra Nevada) was characterized by a general low level of mortality in ponderosa pine. This area was chosen to determine if the low rate of mortality caused by bark beetles was associated with corresponding low incidence of root disease.

Each area was searched for recently killed trees, i.e. trees in the process of fading to those judged to have been dead for not more than 2 years. Since bark beetles reportedly cause extensive top-killing in white fir, a search for white fir with recently killed tops was also made. The trees thus located were examined for root disease and bark beetles, except for the following: (i) trees smaller than 15 cm diameter at breast height, and (ii) trees obviously injured by recent fires, lightning, logging or road construction.

Presence of bark beetles was determined by chopping bark from the stem. Beetles were identified by examining adults or gallery configurations. Possible contributing factors such as stem injuries, mistletoes, galls, cankers, and needle diseases were recorded. Roots and root crowns were examined for decay, stain, resinosis (especially in ponderosa pine), fungus sporophores, mycelia, zone lines and rhizomorphs. Where necessary to determine presence of disease or the identity of the causal organism, the root system was excavated for as much as 2-3 M from the tree. Whenever the identity of the pathogen could not be confirmed in the field, root or root-crown specimens were collected and isolations made in the laboratory. Extent of colonization by the root pathogens, exclusion of secondary organisms and resinosis (a reaction in living tissue) as well as existing knowledge of pathogen biology were used to judge if infection occurred before or after beetle infestation.

To determine the incidence of root pathogens in the overall live tree population, living trees were examined in the same general areas in which the dead trees were located. The technique for selecting these living trees varied somewhat with area and species. Living ponderosa pines on the Georgetown Divide, Boggs Mountain and Plumas National Forest were randomly selected from the area at large and were not

necessarily near the dead trees. Living ponderosa pines at McCloud Flats were randomly selected from the margins of pine mortality centers. Living white firs were either randomly selected from the margins of mortality centers, or they were nearby trees of comparable size and age to the killed trees. The living trees were examined in the same manner as the dead trees.

RESULTS

The results of the ponderosa pine study (Table 1) show a high degree of association between root diseases and infestation by the western pine beetle, Dendroctonus brevicomis LeC., and the mountain pine beetle, D. ponderosae Hopk. The association was particularly strong at Boggs Mountain and on the Georgetown Divide where 71.2 and 82.8 percent, respectively, of the beetle-infested trees had root diseases. Conversely, few root-diseased trees died without first becoming infested by bark beetles.

Table 1.--Causes of mortality of selected ponderosa pines in four areas of California and the incidence of root disease in Dendroctonus brevicomis and D. ponderosae-infested and living trees

Study areas	Boggs Mt.	Georgetown Divide	McCloud Flats	Plumas N.F.
Area sampled (acres)	500	5,000	10	30,000
No. dead trees examined	63	184	59	78
Bark beetles-no root disease	15	42	30	42
Root disease-no beetles	5	6	0	1
Beetles and root disease	37	127	29	28
No apparent cause of death	5	9	0	7
% beetle-infested trees with root disease	71.2	82.8	49.2	40.0
No. live trees examined	67	106	43	66
No. with root disease	2	0	6	2
% live trees with root disease	3.0	0.0	14.0	3.0

The somewhat lower levels of association between root disease and bark beetle infestations at McCloud Flats and on the Plumas National Forest were not totally unexpected. The trees at McCloud Flats were examined during a major bark beetle outbreak in 1968 when the beetle

population was apparently high enough to cause mortality in many healthy trees. This appeared to be borne out when the examined trees were subdivided according to year of beetle infestation. Of those trees killed in 1966 (a year of relatively low mortality), 79 percent had root disease. In 1967, when tree mortality was increasing dramatically, the association dropped to 60 percent, and in 1968 it dropped to a low of 30 percent.

On the Plumas National Forest, mortality in larger ponderosa pine was very low, as evidenced by the size of the area that had to be searched via roads and logging trails to locate an adequate sample of killed trees (Table 1), and a larger proportion of the examined trees were under stress by competition, poor soil drainage and other factors. On the other hand, the low incidence of beetle-infested trees certainly appeared to be correlated with a correspondingly low incidence of root disease.

Very few of the living trees showed evidence of root disease in three of the areas (Table 1). The higher percentage of living trees with root disease at McCloud Flats reflected their location along the margin of the mortality centers.

Four fungi, Fomes annosus (Fr.) Karst., Verticicladiella wagenerii Kendrick, Armillaria mellea (Fr.) Quel., and Polyporus schweinitzii Fr. were the only root pathogens detected during the study (Table 2). Both F. annosus and V. wagenerii were frequently associated with bark beetle-infested trees, but their relative importance varied greatly among the study areas. At Boggs Mountain, only F. annosus was found in association with the primary bark beetles. On the Georgetown Divide and at McCloud Flats, V. wagenerii was the major pathogen, and no F. annosus was detected in ponderosa pine. The absence of F. annosus in the latter areas was partially an artifact of the study sites. F. annosus is known to occur in ponderosa pine at McCloud Flats and to predispose trees to beetles. On the Georgetown Divide, the study area was confined to forest stands above 1200 M elevation where F. annosus has rarely been observed in ponderosa pine. Below that elevation, F. annosus frequently infects the pine.

A. mellea was detected much more frequently than indicated in Table 2, and it was found in all four areas. In several stands, it was associated with V. wagenerii in pine. However, A. mellea either appeared to be secondary or its role in weakening or killing the larger pines could not be established in most cases. It was recorded as a root pathogen only when the evidence was reasonably conclusive that the fungus had caused extensive disease before tree death or beetle infestation.

Extensive decay by P. schweinitzii was observed in only two trees. This may not be a true indication of its relative importance, however, because of difficulties in detection.

While the western pine beetle and the mountain pine beetle were most frequently associated with root-diseased ponderosa pine, other bark beetles, especially the red turpentine beetle (D. valens LeC.), and flathead borers (Buprestidae) were occasionally observed. However, only the relationship between the western and mountain pine beetles and root disease is included in the data in Tables 1 and 2. D. brevicomis was in

about 80 percent of the beetle-infested trees with root disease; D. ponderosae had infested the remaining 20 percent.

Table 2.--Incidence of root pathogens in bark beetle-infested ponderosa pine in four areas of California

Study areas	Boggs Mt.	Georgetown Divide	McCloud Flats	Plumas N.F.
Total no. of beetle infested trees	52	169	59	70
No. of infested trees with:				
No root pathogens	15	42	30	42
F. annosus	37	0	0	11
V. wageneriI	0	111	29	11
A. mellea	0	14	0	6
P. schweinitzii	0	2	0	0

The data from the white fir study (Table 3) were collected in two distinct areas on the Georgetown Divide: (i) Blodgett Research Forest, and (ii) Ellicott's. The dead trees examined on Blodgett Forest were in small mortality centers of 1-3 trees scattered over a relatively large area, and the living trees of comparable size and age were nearby but not within the mortality centers. At Ellicott's, both the dead and living trees were primarily in one large mortality center. Furthermore, root excavation on Blodgett Forest was more thorough than at Ellicott's. These differences are reflected in the data.

All of the killed white firs in both areas had been infested by the fir engraver beetle, Scolytus ventralis LeC. Of the 35 beetle-infested trees examined on Blodgett Forest, all except one had evidence of root disease. F. annosus appeared to be the primary pathogen in 25 trees, A. mellea in eight trees, and the cause of disease in one tree was not determined. F. annosus was detected in only one of 18 live trees examined on Blodgett Forest.

At Ellicott's, 23 of 29 beetle-infested trees showed evidence of root disease (caused exclusively by F. annosus). No conclusive evidence of root disease was found in the other six trees. On the other hand, all 14 of the live trees that were examined were infected by F. annosus.

Of 13 top-killed white firs examined, S. ventralis occurred in three trees infected by F. annosus, in two trees with extensive A. mellea, and in three trees with porcupine feeding damage in the upper bale. The other five trees had injuries caused by porcupine or other causes but with no evidence of S. ventralis infestation. Thus, root

pathogens were associated with the beetle in top-killed white fir. However, there appeared to be several causes of top-killing, some of which did not involve S. ventralis at all.

Table 3.--Causes of white fir mortality in two areas on the Georgetown Divide and the incidence of root disease in Scolytus ventralis-infested and living trees

Study areas	Blodgett Forest	Ellicott's
Area sampled (acres)	200	10
No. dead trees examined	35	29
Bark beetles-no disease	1	6
Root disease-no beetles	0	0
Beetles and root disease	34	23
No apparent cause of death	0	0
% beetle-infested trees with root disease	97.1	79.3
No. live trees examined	18	14
No. with root disease	1	14
% live trees with root disease	5.3	100.0

DISCUSSION

We believe that our results show a strong association between the occurrence of root pathogens and infestation of both ponderosa pine and white fir by bark beetles. These results confirm our earlier report (6) and that of Partridge and Miller (3).

The incidence of disease was directly related to bark beetle infestation not only with respect to individual trees but on a mortality center and area-wide basis as well. Root pathogens were almost invariably present in "bark beetle" centers with a history of mortality extending over a period of two or more years. While it is well established (5, 8) that bark beetles produce a pheromone(s) that stimulates aggregation of the beetles on trees, the pattern of beetle infestation associated with root disease centers suggests that root pathogens that spread locally from tree to tree also affect the pattern of mortality caused by the beetle.

The so-called endemic level of bark beetle populations over relatively large areas may be strongly influenced by the amount of root diseases present. This is supported by a comparison of the ponderosa pine data from the Plumas National Forest with those from the other areas.

Where the incidence of root disease apparently was quite low (Plumas), the number of bark beetle-infested trees also was low. But when the incidence of disease was relatively high, e.g. Boggs Mountain and Georgetown Divide, the beetle population appeared to be maintained at a moderately high level. If this relationship applies generally, root diseases may play an even more important role in the population dynamics of bark beetles.

For example, if the occurrence of root diseases in a forest provides enough host breeding material for the beetles to maintain a population several times that in a healthy forest, the chances for a bark beetle outbreak may be greatly enhanced during a period of general stress (e.g. draught) on the host.

The question of which came first, root diseases or bark beetles, might be raised. In some cases, it is a difficult question to answer especially with respect to insect transmission of the fungi. Such transmission has been investigated, and there is no evidence that the pine beetles, D. brevicomis and D. ponderosae, or the fir beetle, S. ventralis, transmit any of the root pathogens detected during the studies. Furthermore, the extensive colonization of roots and root crowns of the beetle-infested trees by the pathogens, resinosis in infected tissue and exclusion of blue-stain fungi and secondary organisms from the colonized xylem strongly indicate that the pathogens had infected the trees several months or years before beetle infestation.

Because of problems in detection and confirmation of root pathogens, the incidence of root diseases and their association with bark beetle infestations may be even greater than indicated. V. wagenerii was relatively easy to detect on the basis of the typical stain in the roots and root-crowns. Confirmation of F. annosus was far more difficult in both pine and fir. Isolations were often necessary and sometimes negative even though typical symptoms were present. In addition, F. annosus normally did not invade the cambium in fir as it did in pine, and current studies indicate that it can cause extensive decay in the tap root of fir without being detected in the root crown or lateral roots. Similar problems existed with P. schweinitzii in pine and A. mellea in fir.

There is also the problem of the occurrence of pathogens in roots beyond the area of excavation. While this would not be detected and thus would result in an underestimation of the incidence of disease, its importance with respect to bark beetle infestations may not be great. Other studies have indicated that root-diseased trees normally are not attacked by beetles until the fungi advance at least to the vicinity of the root crown.

We believe that infection by root pathogens often has been obscured by beetle infestations and that these pathogens are responsible for much greater loss than previously recorded. V. wagenerii was not detected on the Georgetown Divide, at McCloud Flats or in the study area on the Plumas National Forest until beetle-infested trees were examined during these studies. Nor was F. annosus considered to be as important and widespread in young growth white fir.

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CURRENT RESEARCH ON FOMES ANNOSUS IN FRANCE

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INTRODUCTION

Fomes annosus has been well known by French foresters for a long time but little scientific work has been published in France except by Moreau and Schaeffer (1960-1962). However, the parasite is of great economic importance in France, especially on Norway Spruce (Picea abies (L) Karst), which is one of the most favoured afforestation species. In fact data from various sources show that the infection level can reach 95%. Other species such as maritime pine (Pinus pinaster Ait.) are damaged by F. annosus but are less threatened. Douglas fir (Pseudotsuga menziensii (Mirb.) Franco) and silver fir (Abies alba Miller) are also attacked but at present the extent of the danger to them is unknown. Our work, which has naturally been undertaken on Norway spruce, has developed in two ways: firstly a determination of the exact pattern of the disease in France, and secondly, research into a method of control for spruce.

ESTIMATION OF THE ECONOMIC IMPORTANCE OF FOMES ANNOSUS

This work is just beginning. In the first phase we are trying to devise a simple sampling method for evaluating losses caused by heart rot, first in a given tree and then in a stand of Norway spruce.

It is intended to investigate the relationship between the diameter of the rot at the butt and its height in the bole; this may well be influenced by the diameter of the tree, its age, and various ecological factors.

We are also researching into a statistical method for sampling stands. This method is being explored in collaboration with the Biometric Service in C.N.R.F. (National Centre of Forest Research) and is based on complete diagrams made in the stands. The sampling method will eventually enable an estimate to be made of the general importance of F. annosus in an area, and it will then also be possible to undertake a study into the general ecology of the desease in spruce.

RESEARCH ON METHODS FOR CONTROL IN SPRUCE

Stump treatment following thinning has only limited usefulness in spruce. We need to make this method practical under all circumstances.

Many forest owners don't approve of stump removal after clear cutting. Therefore, we need to solve the problem of what species to plant after clear cutting a plot of spruce damaged by F. annosus. Generally we advise the substitution of spruce by broad-leaved trees. The owners prefer conifers because of their quicker growth. Our limited knowledge makes the choice difficult.

Spruce grows well under many different conditions and we think resistance to F. annosus will be a possibility. However, this will be a long and difficult problem to solve.

We prefer to study the artificial resistance we can obtain from the immunizing commensals described by Ricard (1970). Our work is divided into three parts: 1) the fungi in the wood of living spruces; 2) the comportment of these fungi in vitro (antagonism towards F. annosus on malt agar and in wood, effect on wood); and 3) study of their action on the live trees (a. growth of the antagonist following inoculation, b. absence of adverse symptoms following inoculation, c. no effect on wood structure, d. efficacy against the spread of F. annosus in the tissue).

A summary of our results follows. Some fungi, Nectria cucurbitula sensu Wollenweber, Phialophora sp., Trichoderma album, Ascocoryne sarcoides, Scytalydium sp. (3 strains) are kept for the inoculation of living trees. We attach a great deal of importance to the three strains of Scytalidium because they have a very interesting and strong antagonism against F. annosus in vitro on malt agar and in wood. Two of them are similar to the "Fy" strain of Ricard, the other is like S. lignicolum.

We have only the results 10 months after inoculation of 3 year old seedlings with a small piece of infected wood (Table 1).

Table 1.--Growth of various fungi in 3 year old spruce seedlings 10 months after inoculation

Fungi	Maximum spread (cm)	
	Towards the top	Towards the base
Scytalidium sp.		
#30	+ 0.2	- 0.1
# 2	+ 0.1	- 0.1
#38	+ 0.5	- 0.1
Ascocoryne sarcoides	+ 0.1	- 0.4
Phialophora	+ 0.1	- 0.1
Nectria cucurbitula	+ 0.1	- 1
Trichoderma album	+ 0.1	- 0.1
Fomes annosus	0	0

The growth of Scytalidium and F. annosus in 15 year old spruce following inoculation with a dart is given in Table 2. Lateral spread is always slow in comparison to longitudinal spread.

Table 2.--Growth of Scytalidium sp. and Fomes annosus in 15 year old spruce various times after inoculation on March 19, 1971

Fungus	Incubation	Maximum spread (cm)	
	(months)	Towards the top	Towards the base
Scytalidium sp.			
#38	8	+ 2	- 2
#30	17	+ 2	- 2.5
Fomes annosus	15	+ 12	- 10

Some combined inoculations (F. annosus + antagonists) were also tried on living trees, but results are not yet available.

CONCLUSIONS AND DISCUSSIONS

This study suggests it is possible to introduce fungi which are antagonistic to F. annosus into woody tissues of spruces of various ages. Spread of the antagonists is slow, however, it is more rapid in the longitudinal than in lateral direction. No studies on wood quality are noted here. We need to examine the behavior of F. annosus towards these antagonists in vivo. Also we need to learn more about protection of the whole stem section, because of the limited lateral movement in our studies. In order to have practical application we need to have many inoculations at the same height unless inoculations in the nursery result in a better distribution of the antagonists.

FOMES ANNOSUS BUTT-ROT OF NORWAY-SPRUCE IN THE ČSSR AND INFLUENCE OF VARIOUS FACTORS ON ITS DISTRIBUTION

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Č S S R

INTRODUCTION

Butt-rot Norway spruce in Czechoslovakia is an important problem, because it causes considerable losses of production volume of timber. The most valuable butt wood is deteriorated by the decay and it is to be discarded as fuel wood. Forest stands suffering from butt rot and included in our investigation indicated fuel wood share from 17 to 35%. Considering the fact that normal share of fuel wood in healthy spruce forests of middel site quality is 3-5%, considerable losses due to rot are obvious, notably if we take into account that Norway spruce in Czechoslovakia shows the main share on the total forest yield.

The main pathogen of this decay is Fomes annosus(Fr.). Other species of fungi we found in infected forests were most frequently honey fungus Armillaria mellea(Vahl/Karst), further Stereum sanquinolentum (A.et.Sch.) Fr., Trametes heteromorpha (Fr.) Pres., Fomes marginatus (Fr.) Gillet., Leptoporus borealis (Fr.) Pilát, Leptoporus stipticus (Pers.)

Quel., Phellinus pini (Thore) Pilát, Osmoporus odoratus (Wulf) Singer, Gloephyllum sepiarium (Wulf.) Karst.

At the search of causes of extensive occurrence of rot it is necessary to consider all influences complexly. For this reason we processed methods for the finding of causes for the variation of the occurrence and intensity of butt rot of Norway spruce according to various factors.

METHODS

From a certain unit we selected all homogeneous forest stands with the Norway spruce representation. An independent record list was prepared for each forest stand. The list contained description of site conditions : growth area, climate, typological classification, elevation above sea, relief, exposition, geological underground, soil type and skeleton, soil depth, humification, consistence, soil structure and moisture, underground water, acidity, lime content, nutrition; description of forest stand: silvicultural system, growing stock, canopy, age of spruce and percentage, mean height, mean diameter, site quality, volume per hectare, development stage, way of the forest stand establishment, harmful factors, state of forest stand, fellings, previous culture, tree species composition in the mixture, real area and volume of forest with damaged growing stock. All these data were transformed into numerical symbols and the material processed by punch card system in the Mathematical Statistical Laboratory of the Forestry and Game Management Research Institute VÚLHM Zbraslav Strnady. The analysis of the investigated factors was divided into two parts :

a) relative occurence of butt-rot without respect to the damage degree

b) damage degree of butt-rot from the point of view of the distribution intensity of the decay in the decay in the forest stand.

The analys was based on the use of mathematical statistical methods, mainly test criterion

$$\chi^2 = \sum_{i=1}^{S} \sum_{j=1}^{T} \frac{(n_{ij} - \varphi_{ij})^2}{\varphi_{ij}} < \chi^2_{\alpha}(f)$$

where n_{ij} = observed occurrence frequency

φ_{ij} = expected (theoretical) occurrence frequency

and on the use of analysis of variance.

MATERIAL

According to these methods we evaluated about 1000 forest stands from the area of Forest Management Unit Cervené Poříčí and Forest Management Unit Horšovský Týnec, prevailingly from the growth area " Sumava Foreland ". This area belongs as to climate to slightly warm and slightly humid and humid region. Most of investigated forests are situated in the zone 400-600 m. above sea on slightly slope terrain. The basic mineral are clay slates and flinty slate, granite and gneiss, quaternary deposits and Permian formation, here and there amphibolite. Dominant soil types are pseudogleys and brown soils, mostly middle deep and deep, with middle resource of nutrients. From the typological point of view the forest stands belong mainly to acid beech oak forests, to acid silver fir beech forests and to silver fir forests. A half of the investigated forest stands is formed by pure Norway spruce, in about one third in mixture with other conifers (mainly Scots pine). In other forest stands we find also inmixture of broadleaved trees, mainly with spruce in clear majority. Forests were mostly established by planting and Norway spruce is in 65% of middle site quality, in 28% in the 7th - 8th site quality interclass and only 7% were in the 2nd and 3rd site quality interclass (Czechclassification). The previous culture was found for 60% investigated forest stands.

RESULTS

The analysis of the occurrence of butt-rot of Norway spruce showed that the infested forest amount 12.7% of investigated forests. The infested forest stands represented 19.7% of the total area and about 25 % of the total growing stock of spruce. The occurrence percentage in the individual age classes is very different. The decay occurrence in the age to 30 years in the investigated region was of accidental character, the occurrence in the age from 30 to 40 years appeared to be important and in the age of more than 40 years highly significant. As the age appeared to be an important factor of the decay occurence variation, a special analysis was made for age class 40-80 years which from the point of view of occurrence frequency was statistically homogenous.

Other highly significant factor was the soil moisture : a significantly higher occurrence of the decay was on soils with alternative moisture than on soil with normal moisture. According to these results we were concentrated on forests with middle moisture of soil.

In this complex we find highly significant differences in the decay occurrence in forest stands of different typological classification, of various site quality and on various terrain relief. Total trend was to more pronounced occurrence of the decay in typological units on badly permeable, unsufficiently aerated soils, in stands of worse quality in the plane terrain. More frequent occurrence was found on podzolized soils.

The analysis of the damage degree of forests by butt-rot indicated high significance of soil moisture, typological classification and site quality of forests as factors influencing not only occurrence but also damage degree due to decay. Besides that there were other very important factors : physiological depth of soil and forest stand formation. The lowest infestation degree was on deep soil. The maximal infestation was in pure spruce forests or in mixed spruce stands with other conifers. It was found that spruce representation and soil type may not be underevaluated in our region. (Results were within the limits of statistical significance.)

DISCUSSION

Studies have been carried out to determine methods for investigation of the influence of various factors on butt-rot of Norway spruce, but some results of the analyses are of wider importance.

Results suggests as general knowledge that the occurrence and degree of tree infestation is increased with age as it is listed also by other authors (Low - Gladman 1962 /3-/). Highly significant influence of alternative moisture is to be probably inportant also in other regions as this is connected with the die-back of roots which may become an infection gate (Rohmeder 1937 /1/, Kellner 1951 /2/). Also physiological depth of soil has similar importance. Soil conditions are partly projected into typological classification, but typological complex is not a sufficient criterion for determination of the influence of site conditions. From forest stand factors a very important influence was shown by site quality of forest stand, forest stand formation and representation of Norway spruce.

The advantage of our method consists in the objective decision about the activities of various factors on the occurrence and intensity of butt-rot. The objectivity conditions assume complex data in extensive survey, use of punch card system and data analysis.

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FOMES ANNOSUS IN AUSTRALIA

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INTRODUCTION

Forest and Timber Bureau statistics reveal that there are approximately 400,000 hectares of coniferous plantations in Australia. The major species planted are Pinus radiata D. Don (about 60%); species of southern pine, mainly slash pine (Pinus elliotii Engelm.) (about 30%); and native conifers, mainly hoop pine (Araucaria cunninghamii Ait. ex. D. Don) (about 10%). During the years 1966-1970 the area of coniferous plantings increased at the average rate of about 28,000 hectares per year. These plantings constitute a valuable resource as supplies of coniferous pulpwood are largely imported. The distribution of coniferous plantations in Australia is summarized in Fig. 1.

The first authenticated collection of Fomes annosus (Fr.) Karst. in Australia was made in the vicinity of Mallanganee, N.S.W. in 1919 (bottom arrow, Fig. 1) (8). The next collection was made in 1964 on the Atherton tablelands of north Queensland (top arrow, Fig. 1) (8). These two locations are separated by approximately 1600 kilometers. A few years after the Atherton collection, a third collection was made in the vicinity of Imbil in south Queensland (middle arrow, Fig. 1).

In all of these reports, sporophores were collected from stumps, but not trees, of the native conifer A. cunninghamii.

Because southern pines are susceptible to F. annosus in their native habitat, our main objective was to determine if an Australian isolate of this fungus is capable of infecting southern pines growing in Queensland. We were also interested in determining if native conifers were being attacked by F. annosus. Interest in this work was enhanced by recent reports that an Australian (north Queensland) isolate of F. annosus was pathogenic to seedlings of southern pine growing under controlled conditions (2, 3).

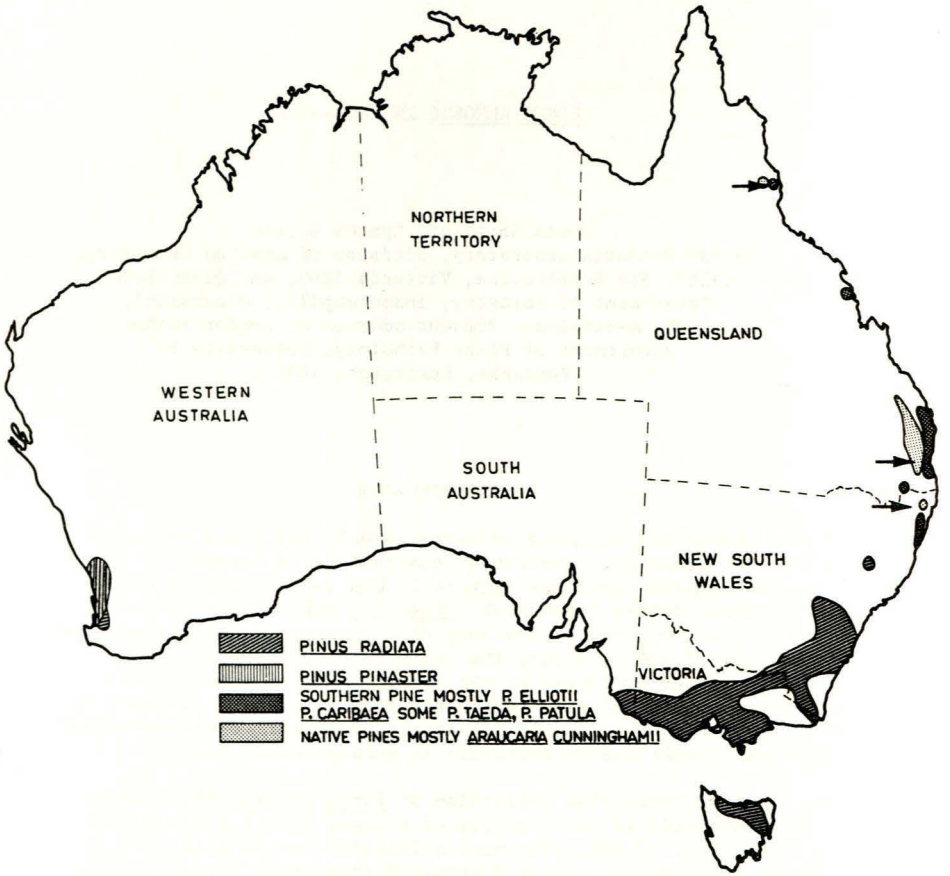

Fig. 1. Distribution of coniferous plantations in Australia. Arrows signify location of authenticated collections of Fomes annosus.

EXAMINATION OF PLANTATIONS

A. South Queensland

In the summer of 1971, thinned plantations of A. cunninghamii (in the vicinity of Imbil) and southern pine, mostly P. elliotii (in the vicinity of Benarkin, Beerburrum, and Beerwah), were examined for the presence of F. annosus sporophores on stumps, dead, dying, and wind-thrown trees. Desiccated sporophores were found only on some stumps of A. cunninghamii in the vicinity of Imbil (middle arrow, Fig. 1). Our inability to locate sporophores on the warmer, drier, sites where southern pine is planted, therefore, could have been due to local weather conditions rather than the absence of sporophores. Attempts to isolate F. annosus from suspect dead and dying trees were not successful.

Subsequent examination of additional plantations in the Imbil area in 1972 revealed sporophores of F. annosus on several stumps of bunya pine (Araucaria bidwillii Hook.). The trees were planted in 1936-37 and the stumps were seven years old. Unhealthy trees were not observed in the vicinity of infected stumps.

B. North Queensland

In the autumn of 1971, a cursory examination was made of the entire plantation of A. cunninghamii (about 12 hectares) from which F. annosus sporophores had been collected in 1964 (top arrow, Fig. 1) (8). Fresh sporophores were present on many of the old stumps resulting either from a thinning in August, 1959 or a salvage thinning after a fire in November 1965. Attempts to culture F. annosus from increment cores from several living trees in proximity to infected stumps were not successful.

In addition to stump infection, sporophores of F. annosus were found on nine standing, dead trees which appeared to have died several years earlier. Seven of these nine trees were broken off from 1-3 meters above ground. Well developed sporophores were present up to two meters above ground. Sporophore production at this height is unusual for F. annosus but, perhaps this is to be expected in the moist habitat of the rain forest.

About a week after examination of the above plantation, A. Bragg of the Queensland Forest Service who assisted in this examination, sent us a sporophore which he collected from a standing, dead Pinus patula (Schlect. et Cham.). We confirmed that the sporophore was F. annosus. This P. patula plantation, established in 1929 on about 2.5 hectares, is about 40 kilometers N.E. of the A. cunninghamii plantation near Atherton. Subsequent examination of this plantation revealed that 320 of the 900 trees present were dead. The dead trees included nine standing trees with F. annosus sporophores and 92 windthrown trees. Only one stump, which was at least five years old, was found in this plantation. This stump was within 4 meters of a dead tree and within 8 meters of a dead tree with a F. annosus sporophore.

INOCULATION STUDY

Since we were unable to detect F. annosus on southern pine in south Queensland, an inoculation study was conducted to determine if a south Queensland isolate of F. annosus could invade the roots of slash pine growing under field conditions.

The slash pine plantation selected for this study is in the vicinity of Imbil and it is adjacent to a plantation of A. cunninghamii containing stumps infected by F. annosus. The isolate of F. annosus selected for study was shown to be pathogenic to slash pine seedlings by a method described previously (6). Inoculum was prepared by placing slash pine dowling (1 X 2 cm) in contact with F. annosus mycelium for about 14 weeks. Histological tests at the end of this period showed that the fungus had permeated the inoculum dowels and had caused advanced decay in earlywood.

In June, 1971, inoculum dowels were inserted into holes drilled in 27 roots of 10 dominant or codominant trees (two or three roots per tree). Sterilized dowels were inserted into six roots of two trees (three roots per tree) to serve as checks. The inoculation technique was similar to that described previously (7). Holes were plugged with sterilized corks and sealed with asphalt paint. A small piece of polyethylene was placed over the point of inoculation before the root was covered with soil.

Isolations [made on modified Russell's medium (4) and malt extract agar] from roots harvested 4, 9, and 15 months after inoculation were negative for F. annosus. Furthermore, F. annosus was not re-isolated from inoculum dowels which usually were resin soaked upon recovery from inoculated roots. The isolation results did not suggest that failure of this isolate of F. annosus to survive and invade roots was due to competition from other microorganisms which may have entered the infection court. These results suggest that products which accumulated during the host response (7) contributed to the failure of this fungus.

DISCUSSION

To our knowledge, this paper contains the first reports of F. annosus occurring on:

i. A. bidiwillii (stumps only).

ii. standing, dead trees of A. cunninghamii.

iii. P. patula (standing, dead trees).

The occurrence of F. annosus on standing, dead trees of Araucaria and Pinus is of particular interest. In view of the poor competitive ability of this fungus in other regions of the world (1, 5) it is quite possible that F. annosus was the primary invader of these trees. Results of the root inoculation study, however, suggest that the isolate tested is not strongly pathogenic under field conditions. This is in agreement with tests of an Australian isolate of F. annosus on seedlings of southern pines (3).

Additional observations and/or testing therefore will be necessary to establish if an Australian isolate can attack native or exotic conifers growing under field conditions. Examination of the above mentioned plantation of P. patula during a thinning operation may be the most expedient means to resolve this question. If F. annosus caused some of the excessive mortality in this plantation, it probably is still attacking living residual trees. Isolation of F. annosus from decayed butts of living trees exposed during the proposed thinning would constitute sufficient evidence that an Australian isolate can attack living trees. Similar observations would be appropriate in stands of native conifers where sporophores were observed on stumps and/or standing trees. Additional artificial inoculations of roots and stumps also may yield useful information.

Although F. annosus appears to have a wide distribution in the rainforest of eastern Australia, it has not been reported in the extensive plantations of P. radiata in southeastern Australia (Fig. 1). Considering the current and projected values of coniferous plantations in Australia, and the increasing number of plantations being thinned, the F. annosus situation warrents additional study and surveilance.

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THE EFFECT OF FOMES ANNOSUS INFECTION ON DIAMETER GROWTH OF PINUS TAEDA L.: AN INITIAL REPORT

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INTRODUCTION

Important measurements of disease severity as caused by Fomes annosus (Fr.) Karst are growth rate reduction and mortality of the host. Economic losses to date have been determined exclusively by measuring mortality. In eastern white pine (Pinus strobus L.) plantations, Boyce, Jr. (3) reported that 12% of the stems died over a period of three years. Powers and Boyce, Jr. (9) found that 75% of slash pine (Pinus elliottii Engelm) stands had general to occasional lethal infections. Powers and Verrall (10) reported 2.8% of the trees dead or dying with some thinned plantations having 30% mortality. Cordell (6) reported mortality losses due to F. annosus in six white pine plantations over a six-year period to be 1.19 cu m/0.4/year. In older plantations, 2.87 and 2.68 cu m/0.4 ha/year were dead. A 20% volume loss over a five-year period due to mortality has been reported in thinned slash pine plantations (7) and a five tree/0.4 ha/year loss in thinned shortleaf pine plantations has also been noted (2). Ross (11) has found up to 5.2% volume loss in slash pine after five years and 11.6% of the volume infected with F. annosus. Morris (8) has reported a loss of 1.81 cu m/0.4 ha/year for a period of nine years following initial thinning for loblolly pine (Pinus taeda L.) plantations in the Coastal

Plain of Virginia on high hazard sites. In this study, the Parker plantation had a loss of 1.45 cu m/0.4 ha/year. These data were based entirely on mortality. Burdekin (4) has suggested the possibility of growth loss in F. annosus infected Scots pine (Pinus sylvestris L.).

The purpose of this investigation was to determine what possible impact F. annosus may have on the diameter growth of infected trees on two different hazard sites.

MATERIALS AND METHODS

Three 0.08 ha plots each on high and low hazard sites were established, in thinned loblolly pine plantations. The plantations were located in the Cumberland (Cu-1) and Buckingham (B-1) State Forests, and Cox (C), Parker (PH and PL), and Union Camp (UC) plantations. The average age of the plantations was 32 years (31 to 36 years). The diameter of each tree was measured at 1.4 m height (DBH) and recorded. Incidence of F. annosus was determined by sampling two roots (45 cm long) from each tree and making root chip isolations onto selective medium (1).

In the Parker plantation, where there were high (PH) and low (PL) hazard plots, five trees geometrically distributed within each high and low plot were selected and their root systems excavated. The incidence of F. annosus was also determined by root chip isolation for these trees.

The average heights of dominant and codominant trees were determined utilizing a site index of 80 which was found to be the average for the six plantations. DBH and height of infected and noninfected trees were used to determine the volume produced (5). The difference between the volume produced by infected and noninfected trees was determined to be the growth loss due to infection. Only dominant and codominant trees were utilized in each plantation.

RESULTS AND DISCUSSION

The greatest growth loss was found in the Cu-1 low hazard plot with 76.02 cu m difference between infected and noninfected trees (Table 1). The low hazard plot (B-1) had a 3.55 cu m loss; Cu-1 and B-1 had a 25% and 8% F. annosus infection, respectively. In the high hazard plantations, C had 40% infection with 14.48 cu m total loss and UC had 31% infection with 9.05 cu m total loss.

Assuming that infection occurred shortly after first thinning, the average volume loss/0.4 ha/year, for the low hazard plantations Cu-1 and B-1 would be 5.83 and 0.22 cu m, respectively. For the high hazard plantations C and UC, the average volume loss/0.4 ha/year would be 1.12 and 0.76 cu m, respectively.

Growth loss of F. annosus infected loblolly pine in PH and PL plantations as compared to different levels of infection are found in Table 2. Using the two root method (TRM), the percent infection was 36% and none for PH and PL, respectively. Total volume loss and

average volume loss/0.4 ha/year since first thinning for PH were 6.88 and 0.54 cu m, respectively. When the all root method (ARM) of sampling was used on five trees in the PH and PL plots, the percent infection increased to 54 and 16, respectively. With these levels of infection, the total volume loss and volume loss/0.4 ha/year since first thinning for PH and PL were 10.32 and 0.80, and 10.72 and 0.83 cu m, respectively.

The results in the Parker plantation demonstrate the importance of utilizing the most accurate method for determining disease incidence because infected trees which are not found to be infected will adversely affect the accuracy of any growth loss data.

These findings indicate that a substantial loss in diameter growth may be occurring in F. annosus infected trees--at least in these plantations. Although mortality has been shown to be very important on sandy soils, diameter growth loss may also be important, especially on clay soils where mortality generally does not occur. Losses in diameter growth of 0.36 cu m/0.4 ha/year and greater are significant. Growth losses in this study were found to be significant with a range of 0.22 to 5.83 cu m/0.4 ha/year for low hazard sites and 0.76 to 0.83 cu m/0.4 ha/year for high hazard sites.

Table 1.--Growth loss based on differences in DBH between infected and noninfected trees on high and low hazard plots.

Plot	Number of Trees	Percent Infection	Growth Loss (Cu m)	
			Total	Since First Thin. 0.4 ha/yr.
Low Hazard				
Cu-1	20	25	76.02	5.83
B-1	11	8	3.55	0.22
High Hazard				
C	10	40	14.48	1.12
UC	13	31	9.05	0.76

Table 2. Growth loss based on differences in DBH between infected and noninfected trees on high and low hazard plots within the Parker Plantation.

Plot	Number of Trees	Percent Infection	Growth Loss (Cu m)	
			Total	Since First Thin. 0.4 ha/yr.
High Hazard				
TRM[1]	11	36	6.88	0.54
ARM[2]	11	54	10.32	0.80
Low Hazard				
TRM[1]	12	0	0	0
ARM[2]	12	16	10.72	0.83

[1]Two roots sampled per tree.
[2]All roots sampled on five trees per plot, TRM on remaining trees.

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DISCUSSION OF

QUANTIFICATION OF LOSSES AND ECONOMIC IMPORTANCE

John Rishbeth, Chairman

Parmeter: Presented paper entitled "Fomes annosus as a factor in the management of recreational forests."

McAree: Can anyone be held responsible for accidents that occur as a result of root rot?

Parmeter: The Park Service is only responsible if it knows about a hazard or can anticipate it. In most cases trees fall without warning.

Koenigs: How did infections originate in the area?

Parmeter: Earlier there was much logging in the valley. Constructional work involved major disturbance to trees, and bark beetle activity was controlled by felling trees.

Krebill: Has a conversion from Pinus to Quercus begun?

Parmeter: No, there has been difficulty with regeneration of oaks. It is not clear what can be grown in the infection centres, nor how long F. annosus might survive in very large pine stumps.

Skelly: What soil types are there in the area?

Parmeter: Generally the soil is a light sandy loam and is well drained; trees are under no water stress.

Russell: Is stump treatment with borax effective, or can F. annosus be found growing down into the stump after treatment?

Parmeter: It is too early to tell; aerial photographic surveys show that infection centres are still arising. It is difficult to investigate fungal colonisation of stumps in the Park itself.

Wallis: What techniques are used to detect root infection?

Srago: Decline in cedars is shown up well with Ektachrome film, but this is not so useful with pines which often fall before they can be detected as being diseased.

Cowling: Borax is being used against new infections but a systemic treatment is needed for established ones: is anyone working in this field?

Parmeter: No work is in progress but it is hoped to investigate the possibility soon. Since trees in the area are very valuable this approach is justified; however, because they are so large it may be difficult to find a treatment that is effective in the roots. Benomyl has been used with nursery stock but there is no report of its effectiveness against F. annosus.

Driver: I should like to add that the U.S. Park Service can spend more money on protecting resources than any other forest management organisation, because it is charged with people's safety and maintaining the condition of the Parks.

Dimitri: Are roots growing directly downwards the ones mostly infected?

Parmeter: No, with pines the laterals are most affected, deep rooting being restricted by the high water table. However in cedars the tap root is often also decayed.

Russell: Ponderosa pine, with roots extending many metres, seems particularly suited to transfer disease: has rooting habit been studied in relation to disease?

Parmeter: Not specifically. The pattern of spread is difficult to understand because a tree may fall some 25 m away from the nearest discrete centre. The possibilities for excavation in the area are very limited.

Rishbeth: Shaw at Oregon State University has recently studied spread of Armillaria mellea on lateral roots of Ponderosa pine.

Sylvestre: Is it known how rapidly F. annosus spreads in the area?

Parmeter: Fortunately in the late 1930s every tree and stump was mapped. Assuming that some of these old stumps lie at the centre of large disease gaps, the rate or spread can be estimated at 1-1.5 m per year.

Alexander: Does the high water table referred to ever reach the surface?

Parmeter: No, only to within a metre or so.

Cobb: Presented paper entitled "Root pathogens as agents pre-disposing Ponderosa pine and white fir to bark beetles."

Russell: Dr. Parker in Idaho uses explosive to blast soil away from root systems as an aid to root-rot research: has this method been tried?

Cobb: Yes, it was tried earlier and is potentially useful, but conditions are hardly suitable in the Park area. For young trees hand winches have proved very useful for extracting root systems.

Yde-Andersen: Is the disease confined to special areas or particular years?

Cobb: Verticicladiella occurs primarily in the central Sierras at a height of 1200-1800 m. By contrast F. annosus occurs on white fir at almost any altitude, though it rarely crosses over into pine from white fir.

Yde-Andersen: In Denmark there was a serious outbreak of F. annosus in Scots pine growing on dry sandy soils in 1947. Mycologists and entomologists both claimed credit for this attack!

Cobb: Entomologists in California now often admit that most of the research should be done on the fungus.

Skelly: In view of the association between F. annosus and beetle attack, do you think that the fungus is carried by beetles?

Cobb: Observations on white fir suggest that infection sites on roots may be associated with insect wounds, but no such relationship has been seen in pines yet. We have no evidence that F. annosus is carried by beetles.

Skelly: In Virginia we found that 9% of black turpentine beetles emerging from infected trees carry the fungus, but we have not demonstrated transmission. Possibly F. annosus dies in the faecal pellets before the beetles reach infection courts.

Myren: In Ontario the rapid killing of red pines infected by F. annosus seems to be due to attack by Ips and associated blue-stain fungi. Do you also find these fungi?

Cobb: Yes, this situation occurs in California: once beetles enter a weakened tree it never recovers. Blue-stain fungi are present and are probably carried externally on the beetles.

Wallis: What percentage of the root system must be killed before beetle attack is successful?

Cobb: With pines F. annosus must have reached the base of one or more roots.

Parmeter: In the case of white fir there is no good way of estimating the extent of root infection. The most important factor seems to be the proportion of conducting tissue still operational. A considerable area of this tissue may be lost as a result of attack: by the time the conducting sapwood has been reduced by decay to a band only a few millimetres thick, bark beetles are very likely to enter.

Redfern: Do you find situations where attack by bark beetles is limited to regions of the stem directly above a one-sided root infection?

Cobb: Yes, in such cases attack by beetles is limited to a strip above the infected roots despite their attempts to enter the whole stem.

Redfern: What is the diameter of the young second-growth trees?

Cobb: Usually it is in the range 15-100 cm, but occasionally it reaches 150 cm. Partial killing is not confined to large trees; it may occur on ones only 30 cm in diameter.

Sylvestre: Presented paper entitled "Current research on Fomes annosus in France."

Skelly: Have you estimated these losses in monetary terms?

Sylvestre: No, but it is planned to do this later. In 30 year-old trees we have found as many as 98% decayed.

Azevedo: Are these stands of the first rotation, and will spruce be planted again?

Sylvestre: Yes, they are, and it is planned to re-plant with spruce.

Parmeter: How is fuel wood selected from the stand; are dead trees used?

Sylvestre: No, they are not dead but extensively rotted.

Greig: What is the soil type in this forest and what was the previous history of the land?

Sylvestre: The soil is calcareous and the land was formerly arable.

FOMES ANNOSUS BUTT ROT. LOSSES IN A YOUNG SECOND ROTATION STAND OF SITKA SPRUCE

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SUMMARY

In a study of butt rot losses caused by Fomes annosus in Sitka spruce, 29% of the trees in one plot were found to be infected 24 years after planting. Volume losses per ha ranging from 3.4% to 9.9% were calculated on the basis of rejection of three categories of butt rot. It is suggested that in normal forest practice these losses would not exceed 5%. The volumes of the butt rot columns in the stems were calculated and found to constitute up to 50% of the total volumes rejected. The significance of the rate of decay development is discussed.

INTRODUCTION

Fomes annosus does not appear to be a major cause of rot in natural stands of Sitka spruce (Picea sitchensis) in North America. In a study of 1414 decayed trees 20 to 750 years old in British Columbia, Bier, Foster and Salisbury (2) found under 1% of the trees studied had F.annosus butt rot. Wright and Isaac (19), working with

Sitka spruce and other species in Washington and Oregon, found that while F.annosus was a leading wound coloniser of Western hemlock (Tsuga heterophylla) it was rare in Sitka spruce. Kimmey (9) found that F.annosus was responsible for only 1% of the total rot cull in Sitka spruce in Alaska.

In Europe, the tendency of plantation-grown Sitka spruce to develop F.annosus butt rot is well established. Early work in Denmark by Løfting (12) and Jørgensen, Lund and Treschow (7) recognised the susceptibility of Sitka spruce to both butt rot and killing attacks. Peace (13) found 21% and 48% infection in two crops aged 16 and 21 years in Wales. Francke-Grosmann (3) commonly found up to 40% of trees in 40 year old plantations attacked by F.annosus. Low and Gladman (11) reported 21% infection in a Sitka spruce crop in Scotland, and suggested the species might be more susceptible to butt rot than Norway spruce (Picea abies). Holmsgaard, Neckelmann, Olsen, and Paludan (5) compared the susceptibility of Sitka spruce and Norway spruce to F.annosus butt rot in 14 paired plots in Denmark, and concluded that Sitka spruce was generally the more frequently attacked.

Apart from these reports, there appears to have been little research done on F.annosus butt rot in Sitka spruce. Butt rot loss studies in Europe have been mainly confined to Norway spruce (e.g. Arvidson (1), Rattsjö and Rennerfelt (14), Kallio (8)).

Sitka spruce was introduced into Great Britain in 1831. It was first planted extensively in the 1920's and is now commercially the most important coniferous species, occupying 37% (223,500 ha) of state forest land. In 1971, 30% of the softwood production from state forests was Sitka spruce, and it is likely that this proportion will increase to 40% during the next 20 years. Most Sitka spruce plantations in Britain are in the higher rainfall areas of the North and West, where it appears to thrive. The species also grows well on some of the drier sites in the East, and will probably be used increasingly to replace less economic but traditional crops of Scots pine (Pinus sylvestris), European larch (Larix decidua) and Norway spruce on some of these sites. The importance of conifer stumps in the life cycle of F.annosus was established by Rishbeth (15, 16 and 17) and, notwithstanding current stump protection practice, it is likely that replacement of these species by Sitka spruce will result in F.annosus attack and subsequent butt rot on some of these sites, especially on those thinned before stump protection was begun.

In 1970, a project was designed to measure F.annosus butt rot in Sitka spruce and to evaluate the maximum losses that could be expected to occur in heavily infected second rotation stands aged 20 to 50 years. The data from the

project will also be used as a basis for calculations on the costs and benefits of a number of remedial treatments for heavily infected stands. So far, twenty-three study plots have been laid down in infected stands throughout Britain. In this paper, the preliminary results from one study plot are presented and discussed.

MATERIALS AND METHODS

A standard method of evaluating losses was devised and has been used in all plots. The method relies on the assumption that losses are dependent on the total rejection of infected timber lengths containing up to three stages of butt rot. These stages, which can be recognised in the forest, are:

Stain. Wood structure not visibly altered; wood discoloured pink to purple. Stain is often considered an early stage of butt rot; it may indicate the onset of decay in a recently infected tree, or it may commonly be seen in advance of, and surrounding, columns of incipient decay and decay in the stems of infected trees. It is not always fungal in origin, and may sometimes be caused by reaction in the stem to infection. Nevertheless, as long as it is associated with F.annosus, it may be considered a legitimate butt rot defect.

Incipient decay. Wood firm, brown to yellow in colour often with some straw-coloured 'flecking'. Incipient decay is an intermediate stage between stain and decay. Break-down of cell walls can be distinguished under the microscope, and it is probable that some reduction in the strength properties of the timber has occurred.

Decay. Wood structure is visibly altered, with obvious break-down of cell structure. Wood firm to soft, generally reddish-brown in colour. The term decay covers all stages of decay, from early 'pitting' to advanced decay with total destruction of the wood.

These stages of butt rot are similar to those described independently by Roeder (18) for measuring losses in Norway spruce. The typical pattern of butt rot consists of a series of concentric columns or cones, each containing one or more stages of decay or stain, and are similar to those found in Norway spruce.

The volumes of the butt rot columns, and the volumes of the rejected timber lengths in which they occur, were calculated from a series of cross-sectional measurements

of the surface areas of stain, incipient decay and decay taken at 0.5 metre intervals up the stem, the trees having been cross-cut at these points to determine the lengths of the butt rot columns.

Losses are expressed as the difference between the gross merchantable volume of a stem, and the net merchantable volume remaining after rejection of butt-rotted timber lengths. Three losses were calculated for each tree, assuming increasingly severe criteria for rejection of butt-rotted timber lengths:

I. rejection of decay;
II. rejection of incipient decay and decay;
III. rejection of stain, incipient decay, and decay.

This study was made in a 24 year old stand of Sitka spruce, in Dunkeld Forest, central Scotland. Elevation of the stand is 150m above sea level, and annual rainfall is 1100mm. The soil is fertile and well drained, and is similar to the Mesotrophic Brown Earth described by Kubiëna (10). It has developed over Dalriadan Shistose-grit, and has pH 3.8 at 150mm and pH 4.6 at 700mm.

The first conifer plantation on this site was of European larch, planted in about 1890 following abandonment of agriculture in the area. When the larch was felled in 1945, F.annosus was causing some decay, and is still active in some of the remaining stumps. The Sitka spruce was planted in 1947, at about 1.5m spacing. Early growth was rapid, and the crop is now in a high yield class of 24. It was first thinned in 1968.

A plot containing 100 trees was demarcated in the stand in an area where F.annosus was found to be present. Each tree in the plot was numbered, and its diameter at breast height (dbh) was measured. The dominance of each tree was assessed using standard Forestry Commission procedure (6). The standing volume per hectare was calculated. Stand mensurational details are given in Table 1.

Table 1.--Stand mensurational details (1971)

Stems ha	Mean dbh (cm)	Mean Timber height (m)	Mean ann. increment (cu m./ha)	Mean tree volume (cu m.)	Standing volume (cu m./ha)
2,500	17	9.9	24	0.13	325.0

Butt-rotted trees in the plot were located by taking one pressler boring from each tree at about 0.75m above ground level. These diseased trees were felled and measured, and the various losses in each tree were calculated as described.

RESULTS

The distribution of butt-rotted trees in the plot is shown in Fig.1.

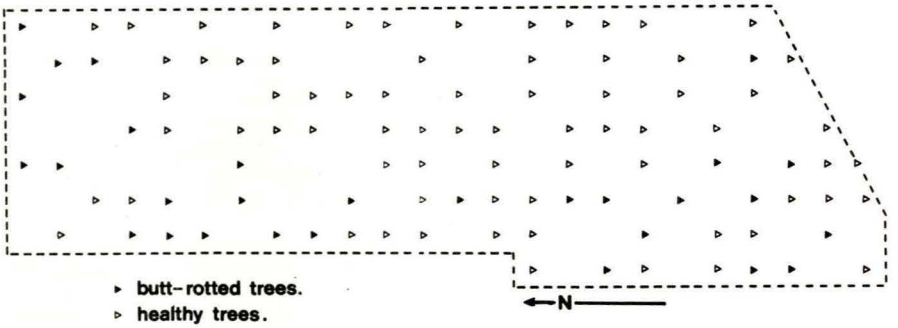

Fig.1. Distribution of butt-rotted and healthy trees in the plot.

Trees with butt rot tend to be concentrated towards the North and West sides of the plot. Statistical analysis of the distribution of butt-rotted trees did not reveal any obvious grouping.

Three categories of disease were noted in felled, butt-rotted trees:

A. trees with stain only (1 tree)
B. trees with incipient decay and stain (9 trees)
C. trees with decay, incipient decay and stain (19 trees).

The number of trees without butt rot (healthy trees), and trees in the three disease categories, are shown in relation to dominance and diameter at breast height (dbh) classes in Tables 3 and 4.

Table 3.--Number of trees in dominance classes, by disease categories

Disease Category	Dominance Classes			
	Dominant	Co-dominant	Subdominant	Suppressed
Healthy	15	31	19	6
A	1	-	-	-
B	-	6	3	-
C	3	7	8	1

Table 4.--Number of trees in dbh classes (cm) by disease categories

Disease Category	dbh Classes				
	28-32cm	23-27cm	18-22cm	13-17cm	8-12cm
Healthy	2	3	26	22	18
A	-	-	1	-	-
B	-	-	3	4	2
C	1	1	5	7	5

No significant association between incidence of diseased trees and either dominance or dbh classes was revealed using a chi-squared test on these data.

The longitudinal sections of butt-rotted trees in Fig.2. are taken from drawings made by computer from data collected from three trees of approximately equal size from this plot. The drawings show the typical development of butt rot in trees of the three disease categories, from the early stage where initial infection probably occurred recently and is limited to stain at the butt, through an intermediate stage of incipient decay and stain, to the final stage where infection has been established for some time and decay has developed in the lower portion of the stem.

The lengths of the columns of decay, incipient decay and stain vary widely between individual trees, and range from under 0.5m to 4.5m. Table 5 shows the number of columns in the three stages of butt rot ranged in 0.5m length classes.

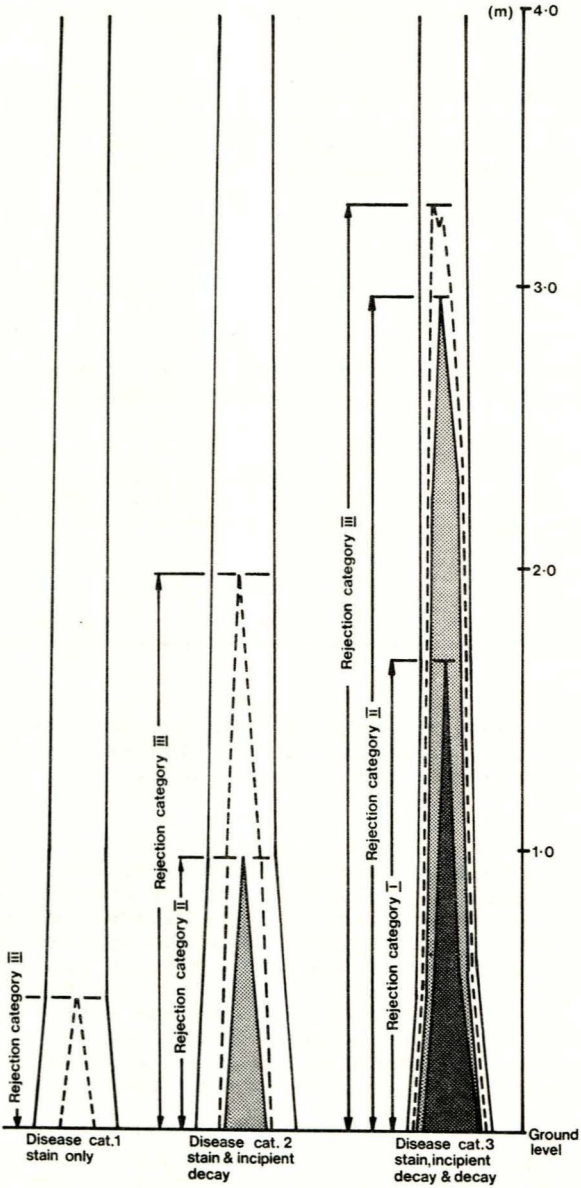

Fig.2. Longitudinal sectional drawings of butt rot in trees of the three disease categories.

Table 5.--Numbers of butt rot columns classified according to their total lengths

Butt rot stage	Total length of butt rot column (m)								
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Decay	8	7	1	3					
Incipient decay	1	8	6	6	4	1	1	0	1
Stain	1	6	6	7	5	1	2	0	1

The mean length of all the decay columns is 0.8m; all incipient decay columns 1.6m, and all stain columns 1.7m. The highest vertical extent of butt rot of 4.5m was found in a tree with a column of soft, advanced decay 2m long, infection occupying 86% of the surface area of the stump.

The lengths of timber that would be rejected using the three increasingly severe rejection categories are also shown in Fig.2. For example, the tree with decay (disease category 3) would lose 1.7m of butt length if decay is rejected (rejection category I), 3.0m if incipient decay and decay are rejected (rejection category II), and 3.25m if stain, incipient decay and decay are rejected (rejection category III). In comparison, the tree with stain only at butt (disease category 1) sustains no losses when rejection categories I and II are used, and only loses 0.5m under rejection category III.

A summary of the losses that result from rejection of the infected lengths is shown in Table 6. The table shows the mean losses from trees in each disease category, and includes the mean lengths of the rejected pieces, the mean volumes of the internal columns of butt rot (butt rot vol.), and the mean volumes of the rejected pieces of timber (rejected vol.) The volumes are expressed as percentages of the mean volume of the trees in each disease category.

Table 6.--Mean losses classified by three rejection categories for trees in the three disease categories, Volume losses are expressed as a % of the mean volume of trees in each disease category.

Rejection Category	DISEASE CATEGORIES											
	A (Stain only) 1 tree. Vol 0.165 cu m.			B (Incipient decay & stain) 9 trees. Mean Vol 0.137 cu m.			C (Decay, Incipient decay & stain) 19 trees. Mean Vol 0.141 cu m.			Mean of all Disease Categories Mean Vol. 0.141 cu m.		
	length of rejected pieces (m)	butt rot volume (%)	rejected volume (%)	length of rejected pieces (m)	butt rot volume (%)	rejected volume (%)	length of rejected pieces (m)	butt rot volume (%)	rejected volume (%)	length of rejected pieces (m)	butt rot volume (%)	rejected volume (%)
I (rejection of decay)	0	0	0	0	0	0	0.8	2.7	19.6	0.8 (19 trees)	2.7	19.6
II (rejection of incipient decay and decay)	0	0	0	1.0	4.2	18.3	2.1	14.6	37.6	1.7 (28 trees)	11.3	31.4
III (rejection of stain, incipient decay and decay)	0.5	0.6	15.0	1.1	7.5	20.4	2.2	21.9	39.8	1.8 (29 trees)	16.7	32.4

The table shows that the rejected lengths, and therefore the rejected volumes, are greatest in the trees with more advanced butt rot development. (i.e. disease category 3). The maximum mean loss in this category when stain, incipient decay and decay are rejected is 39.8% of the mean tree volume. In comparison, the internal column of butt rot occupies 21.9% of the same mean tree volume, or 55% of the volume of the rejected length. Rejection of infected lengths implies rejection of some sound or otherwise acceptable wood as well, and the figures show that this sound wood can account for between 14% and 20% of the mean tree volume.

Table 7 shows the accumulated losses of all the infected plot trees converted into losses per hectare. The calculation assumes an overall similarity in incidence and severity of attack over an hectare as was found in the plot.

Table 7.--Losses per hectare classified by rejection categories

Rejection Category	Butt rot volume		Rejected volume	
	cu m.	% of total Vol/ha	cu m.	% of total Vol/ha
I	1.2	0.4	10.9	3.4
II	10.4	3.2	29.4	9.0
III	15.5	4.8	32.1	9.9

These figures show that the biggest losses occur from rejection of incipient decay (rejection category II): this accounts for 67% of the total butt rot volume, and 92% of the total rejected volume.

DISCUSSION

The losses described here for the three rejection categories are based on accurate measurements of the vertical limits of butt rot, and assume total rejection of butt-rotted lengths. The volume losses shown in Table 7 therefore probably represent the maximum losses that could be expected now for this site. It is likely that in normal forest practice losses would be restricted to rejection of most of the decay and some of the incipient decay, and would probably not exceed 5% of the total volume. A realistic evaluation of value loss is more complex in that it

would probably include a lower negotiated selling price for all the timber, based perhaps on a small sample felled prior to sale, as well as direct rejection or a reduction in value of unacceptably decayed timber lengths. Prices vary throughout the country, and also depend somewhat on market and seasonal influences, and it is for this reason that no emphasis has been placed on economic losses in this part of the study. However, using the data collected from each plot, economic losses based on a variety of specifications and values can readily be calculated.

If it can be assumed that the stumps from the previous crop were the main source of infection, and that losses have been accumulating as decay and incipient decay in the stems of infected trees since perhaps the fifteenth year, it can be calculated (on a basis of a loss to date of 5%, or 16 cu m./ha) that the average annual loss in the last nine years has been about 1.8 cu m./ha, or 5.6% of the current annual increment of 32 cu m./ha. It is unlikely that these losses are sufficiently high to create immediate management problems. However, the outlook for the crop is less certain, especially if it is to grow to a rotation of 50 years. The future development of butt rot in infected and as yet uninfected trees is not known. It is probable that the current high rates of growth of the crop on this site will not be sustained, especially when the trees become more exposed. A reduction in the growth rate may have the effect of increasing the proportion of butt rot and reducing the critical limits of profitability. In addition, indirect losses caused by windblow of trees with root systems severely decayed by F.annosus will also tend to increase.

An understanding of the speed and pattern of butt rot development is essential to any work which aims to predict losses. Little experimental work on the measurement of growth rates of the fungus in Sitka spruce has been done. Gibbs (4) has shown that growth rates of about 20mm per month were achieved by F.annosus following root inoculation of 40 trees, and he concluded that once infection is established in the roots of Sitka spruce there is little active resistance to the growth of the fungus in the central stemwood. However his was essentially a short-term (14 months) experiment and although it provides valuable data on the speed with which F.annosus can colonize living Sitka spruce wood, it does not indicate the speed of subsequent decay development. Since losses are closely related to the lengths of timber affected, it is important to know the rate at which F.annosus can colonise and decay during a rotation of 50 years, and whether this is independent of tree growth. Similarly it is necessary to know what factors, of the trees or the sites, influence the volumes of timber affected. A study of butt rot development in the stem, rather than a study of gross economic losses, could go some way to answering these questions. Gross economic losses are to some extent dependent upon the

incidence of butt-rotted trees, and it seems probable the full study will show that incidence is the most variable and unpredictable of the factors involved. When sufficient data on disease incidence are collected and consequent losses are calculated, it may be possible to calculate losses for a variety of sites based on theoretical incidences, bearing in mind that the relationships between disease incidence and loss may be complex. For example, Holmsgaard et al (5) showed that in infected populations of Norway spruce, the proportion of badly decayed trees tended to be higher in crops with a high incidence of attack. Ultimately the aim of the full study will be to provide a guide, combining incidence and predictable losses, which can be used by forest managers for the proper management of infected crops and for the best choice of species on sites where F.annosus is a potential hazard.

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FOMES ANNOSUS: MORTALITY RATES IN YOUNG TREES UNDERPLANTED AMONG PINE

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INTRODUCTION

In Britain, killing by Fomes annosus occurs mainly in pines. Rishbeth (6) showed that the development of F.annosus was greatest on calcareous soils, and pines are the conifer species generally planted on such areas. Other species are less commonly killed but Rishbeth (7) reported deaths in European larch (Larix decidua), Douglas fir (Pseudotsuga menziesii), Sitka spruce (Picea sitchensis), Beech (Fagus sylvatica), and Birch (Betula).

Many of the severest attacks of F.annosus in Britain have been in Thetford Forest in Eastern England (2.4.5). This forest consists of 19,000 ha of predominantly pine plantations throughout which F.annosus is almost ubiquitous. This paper

describes the effects of F.annosus in an experiment laid down to assess species that might be planted in the Thetford area as alternatives to pines.

METHODS

The underlying geological formation of Thetford Forest is chalk, but soils vary within the area and can generally be grouped in the following three types -

A - Worlington series (Brown earth - sol lessives) Deep acid sandy soil (> 75 cm in depth) pH about 4.5

B - Methwold/Worlington complex (Brown calcareous soils). Sandy soil of variable depth and pH generally > 6.0

C - Newmarket series (Rendzina). Shallow calcareous soil (< 30 cm in depth) pH about 8.0

Three sites were chosen as being representative of the three soil types A,B and C and on each site the existing crops of Scots pine (30 to 40 years old) were thinned to leave about 500 stems per hectare. Fomes annosus was present in old thinning stumps throughout all three sites. The areas were underplanted in 1964 with 18 species (see Table 1) in 0.04 ha plots. A randomised block design was used to give three replications of each species on the three soil types. (54 plots per site: 3 blocks x 18 species).

Each winter from 1967 onwards, all dead trees were removed from the plots. A record was made of trees killed by F.annosus, the cause of death being confirmed by the presence of sporophores at the base of the trees or on the roots.

RESULTS

General

Atlas cedar failed on all three sites and was discarded from the trial. The growth of Greek fir was poor and only about half the trees survived. The remaining 16 species were all established satisfactorily, although there was considerable variation in growth between them. In the faster growing species F.annosus killed a few trees in the summer of 1965, i.e. in the second season after planting and more deaths occurred in 1966. The first assessment for all the plots was made in 1967 and therefore included a

cumulative element for some of the faster growing species, e.g. Leyland cypress, Douglas fir and Western hemlock.

Table 1.--List of species in the experiment

Species	English name	Abbreviation
Pinus sylvestris L.	Scots pine	SP
Pinus nigra var. maritima (Aiton) Melville	Corsican pine	CP
Pseudotsuga menziesii (Franco) Mirb.	Douglas fir	DF
Tsuga heterophylla (Raf.) Sarg.	Western hemlock	WH
Thuja plicata D. Don.	Western red cedar	RC
Chamaecyparis lawsoniana (A. Murr.) Parl.	Lawson cypress	LC
Cupressocyparis leylandii (Jacks. and Dallimore)	Leyland cypress	Ley C
Larix eurolepis Henry	Hybrid larch	HL
Picea omorika (Pancic) Purkyne	Omorika spruce	OS
Cedrus atlantica Manetti	Atlas cedar	-
Abies amabilis (Douglas) Forbes	-	-
A. alba Mill.	Silver fir	SF
A. concolor (Gordon) Engelmann	-	-
A. cephalonica Loudon	Greek fir	-
A. grandis Lindl.	Grand fir	GF
Fagus sylvatica L.	Beech	Be
Quercus borealis Michx.	Red oak	R. oak
Nothofagus obliqua (Mirb) Blume	-	N. ob

Variation In Killing On The Different Soils

Table 2.--Percentage mortality caused by Fomes annosus eight years after planting on the three soils A, B and C

Species	Total Mortality Percentage Soil A	Soil B	Soil C
Scots pine	6.1	28.1	17.6
Corsican pine	3.1	12.2	16.2
Douglas fir	10.4	45.7	18.3
Western hemlock	3.8	22.2	8.9
Western red cedar	0.6	6.7	8.0
Lawson cypress	4.0	21.7	16.9
Leyland cypress	6.6	51.5	37.0
Hybrid larch	1.4	23.5	7.4
Omorika spruce	1.1	15.1	7.8
Abies amabilis	1.3	17.0	3.3
Silver fir	0.2	12.7	3.0
A. concolor	0.6	14.8	3.5
Greek fir	0.0	2.8	1.2
Grand fir	0.2	7.1	3.4
Beech	1.6	9.7	2.2
Red oak	4.4	25.1	4.2
Nothofagus obliqua	4.1	38.3	21.7
MEANS	2.8%	17.0%	9.7%

Table 2 shows the mean percentage mortality caused by F.annosus up to the end of 1971, eight years after planting, on the three soil types A,B and C. Differences in the losses between sites can only be attributed to site factors if tree growth on the sites is similar, because infection depends, ceteris paribus, on root growth and spread and contact to a series of static infection sources. The mean heights for all species, six years after planting were:

Soil A - 1.68 m
Soil B - 1.70 m
Soil C - 1.06 m

Therefore the differences in mortality between A and B can be attributed to factors other than growth. The cumulative losses to date (eight years after planting) for all species are 2.8% on Soil A, the deep sandy acid soil, compared to a mean of 17.0% for the calcareous Soil B. The losses on Soil B for Leyland cypress (51.5%) and Douglas fir (45.7%) are extremely high and for trees of this age are the heaviest recorded anywhere in Britain. The high losses

in some of the hardwood species, e.g. Nothofagus obliqua (38.3%) and Red oak (25.1%) are also surprising, as although it was known that hardwoods could be killed by the fungus it was generally expected that these trees would be more resistant than most conifers. The relative susceptibility of the different species in the experiment will be discussed later in this paper. The losses on the third site, Soil C, the shallow alkaline soil are so far intermediate between Soils A and B, the mean loss for all species being 9.7%. The mean heights of the trees on Soil C are more than one third less than on the other two soils and the lower losses compared to Soil B probably only reflect this poorer growth. Table 3 shows the number of trees killed by F.annosus on the three soils for each assessment. From the table it can be seen that on Soils A and C the killing has remained fairly constant at each assessment, although almost four times as many trees died on Soil C than on Soil A. On Soil B in contrast 37% (544 trees) of the mortality to date was recorded at the first assessment in 1967; in subsequent years the killing has continued at a high level, although there may now be a diminishing trend as only 130 trees died in 1971, which is less than that recorded in Soil C. The first assessment of all sites in 1967 will have included some trees which died in 1965 and 1966.

Table 3.--Numbers of trees killed by Fomes annosus on the three soils A, B and C

Soil type	Number of dead trees recorded at each assessment					Total Nos killed by F.annosus
	1967	1968	1969	1970	1971	
A	55	42	39	65	52	253
B	544	283	211	310	130	1478
C	196	139	156	187	203	881

Variation in pH of 3.9 to 8.3 exists over the site on Soil B, with the eastern half being in general alkaline with pH's approximately 8.0, whereas neutral or slightly acid conditions occur in the western half of the site. A soil sample was collected from a central point in each plot at a depth of six inches. The pHs of these samples were measured in the Soil Laboratory, using electrodes and a pH meter. Differences in losses between plots of the same species can be broadly linked to the variation in pH. Because of this from the three plots of each species the highest and lowest loss percentage were taken and the pHs for these plots compared (see Table 4). With thirteen species the lowest loss was from the plot with the lower pH of the two. The only exceptions were Beech,

Red oak and Douglas fir. For Beech and Red oak the pHs of the two plots were similar and losses did not differ appreciably. With Douglas fir, although the recorded pH for the plot with the highest mortality was low, most of the losses occurred in part of the plot that was extremely alkaline. The mean mortality for the plots with the lowest killing is 16.06% against 29.09% for the highest and the pH value for the lowest plots is 5.95 compared to 7.63 for the highest. The differences between both groups are highly significant, in terms of both pH and mortality. The variation in pH is very local and changes occur within a few metres and occasionally these changes are reflected in killing within a single plot. For instance, in one Leyland cypress plot all the deaths have occurred in the northern alkaline half of the plot and in the acid southern half no deaths have occurred.

Table 4.--Comparison of mortality and pH on Soil B

Species	Plot with lowest mortality % Loss	pH	Plot with highest mortality % Loss	pH
Scots pine	17.8	5.80	39.9	8.05
Corsican pine	7.7	5.60	22.0	6.95
Douglas fir	38.2	7.65	53.7	5.50
Western hemlock	16.4	5.20	25.0	8.05
Western red cedar	5.9	4.75	6.9	8.00
Lawson cypress	19.0	4.45	35.3	7.85
Leyland cypress	30.4	5.02	63.3	8.05
Hybrid larch	15.3	7.55	33.6	8.15
Omorika spruce	8.6	5.30	18.8	8.20
A.amabilis	6.2	7.85	26.4	7.90
Silver fir	5.5	6.30	16.3	8.00
A. concolor	8.9	6.50	25.6	8.10
Grand fir	5.4	4.10	12.4	7.80
Beech	11.8	7.90	13.3	7.55
Red oak	21.5	6.40	27.1	6.15
N. obliqua	38.3	4.85	45.9	7.85
MEANS	16.06	5.95	29.09	7.63

NB Greek fir omitted as only 2 trees died on the site.

Significance 0.01 between pH groups
Significance 0.1 between Mortality groups

Variation In Species Susceptibility

Although there are large differences in the losses between species in this trial (see Table 2), the results must be interpreted with caution in terms of species susceptibility. The species included in the trial differ in their rates of growth and suitability to the conditions in Thetford. There are large differences in heights and therefore also presumably in root spread between the species. For example on Soil B mean heights range from only 0.67 m for Silver fir to 5.37 m for Leyland cypress and therefore although the losses in the former are only a quarter of losses in the latter, Leyland cypress may not necessarily be more susceptible than Silver fir. For when the Silver firs reach the equivalent height of the Leyland cypress the losses may be as great. So we can only say on this site at eight years after planting, the Leyland cypress are more susceptible than Silver firs. In order to make better comparisons between species various analyses were made of the results from Soil B, where the highest losses caused by F.annosus occurred. The height and stem diameters at ground level for each tree killed by F.annosus was recorded and averaged for each species. The regression of current and cumulative Fomes killing percentage (mean for three plots) for 1968, 1969, and 1970 against the three factors of Height, Stem diameter and Height x Stem diameter was calculated. The independant variable that gave the best fit was chosen as the covariate in an analysis of covariance. Duncan's multiple range test was subsequently applied to the adjusted mortality means for the species. From Table 5, which shows the 1970 cumulative results, it can be seen whether any particular species is more or less susceptible or has no significant difference from any other. For example when Scots pine and Hybrid larch are grouped together, they are more susceptible than Beech, Corsican pine, Grand fir and Western red cedar, are less susceptible than Leyland cypress and do not differ significantly from the other nine species in the trial.

Table 5.--Susceptibility of species to killing by Fomes annosus on Soil B

Species group	More susceptible than	Less susceptible than	No significant difference from
Leyland cypress	SP, HL, R.oak, LC, WH, A.amabilis, OS, A.concolor, SF, Be, CP, GF, RC.	-	DF, N.obl.
Douglas fir) Nothofagus obliqua)	R.oak, LC, WH, A.amabilis OS, A.concolor, SF, Be, CP, GF, RC.	-	Ley.C, HL, SP.
Scots pine) Hybrid larch)	Be, CP, GF, RC.	Ley.C.	DF, N.obl, R.oak, LC, WH, A.amabilis, OS, A.concolor, SF.
Red oak Lawson cypress Western hemlock A.amabilis Omorika spruce A.concolor Silver fir	-	Ley.C, DF, N.obl.	SP, HL, Be, CP, GF, RC.
Beech Corsican pine Grand fir Western red cedar	-	Ley.C, DF, N.obl, SP, HL.	R.oak, LC, WH, A.amabilis, OS, A.concolor, SF.

For abbreviations see Table 1.

DISCUSSION

A number of new hosts for F.annosus occur in this experiment. Additions to the list of Sinclair (8) are Greek fir (Abies cephalonica), Leyland cypress (Cupressocyparis leylandii), Nothofagus obliqua, Omorika spruce (Picea omorika) and Hybrid larch (Larix eurolepis). Hybrid larch, although not included by Sinclair, had previously been recorded by Peace (3), who found Hybrid larch trees with butt-rot caused by F.annosus.

Wallis (9) from his survey of East Anglian pine plantations, mainly first rotation areas at least twenty years old, considered that killing by F.annosus was severe only on soils with a pH greater than 6. This present experiment confirmed that severe attacks of the disease are restricted to the calcareous soils, at least for young trees up to the age of eight years. On the acid soils losses are very low in comparison to those occurring on calcareous soils and do not appear to be increasing (see Table 3). On the brown calcareous soils (e.g. Soil B) where early tree growth is good, the young, small trees appear to be very susceptible, for mortality rates are extremely high in the first few years and trees die within a few months of attack. As the numbers of surviving trees are reduced and the trees become larger and more resistant, at about eight or ten years of age, it is expected that current annual losses will fall and be less than mean annual losses. However, in the experiment, stabilisation of killing has not yet been reached as deaths are still occurring even in Leyland cypress and Douglas fir, the species with the highest losses.

The results show that on the alkaline areas, attack can be extremely severe with several species and losses exceeding 50% may be expected. Serious consideration should therefore be given to eradication of the disease from these sites, as proposed by Greig and Burdekin (1) before new crops of susceptible species are planted. Although killing by F.annosus is not a problem on the acid sites, damage by the disease will occur on these through butt-rot and root-rot, particularly in the case of the more susceptible species. Therefore it may not be possible to choose the species otherwise best from a silvicultural point of view, because of likely future losses through butt-rot. For example, the growth of Western hemlock is superior to all other species on the acid Soil A and silviculturally is an excellent choice for underplanting and on this site is not particularly

susceptible to killing, but this species is extremely susceptible to butt-rot by F.annosus (2). Species may differ in their susceptibility to butt-rot and killing, e.g. pines are very susceptible to killing by F.annosus, but are relatively resistant to butt-rot. Root-rot will also be severe with many species (e.g. Douglas fir) and make the trees liable to wind-throw. Assessment of these trials will continue and they will be used in the future to measure differences in susceptibility to butt-rot between the species.

Underplanting gives the young trees protection against spring frosts by leaving part of the original crop standing. Losses caused by F.annosus in the underplanted trees may be greater than with clear felling and immediate replanting. For with underplanting the overstorey is normally felled six to ten years later and the resulting stumps are then an additional source of infection for the young trees. Fomes annosus can remain active in pine stumps for at least twenty years and therefore the trees remain at risk for a longer period than with clear felling.

The susceptibility of species to killing by F.annosus can be considered in two ways. Firstly the species can be ranked in order from highest to lowest mortality. Comparison of ranks made from Table 2, for Soils B and C, give reasonable agreement, with Leyland cypress, Douglas fir, N. obliqua and Scots pine being the four species with the highest losses for both soils. This method has the advantage of giving the current losses, but may not indicate future trends. Secondly an attempt can be made to compensate for differential growth between the species by adjusting mortality using a measure of growth factors. This method is theoretical and its validity will be tested by the use of future results. It may prove to give a truer indication of the long-term susceptibility of the species than does a simple comparison of current losses.

In considering the losses caused by F.annosus in these trials, it should be emphasised that the infection sources were stumps of Scots pine, which are usually much more completely colonised by F.annosus than are those of other species. Therefore losses might be less for instance among trees planted under larch or even under Corsican pine. Wallis (9) suggested that losses in Corsican pine were greatest when root contact was with Scots pine stumps and much less when only Corsican pine stumps were involved.

To summarise, it appears that Leyland cypress is outstandingly susceptible to killing by F.annosus and therefore should not be planted on calcareous soils where F.annosus is infecting stumps. Douglas fir and Nothofagus obliqua are also extremely susceptible to F.annosus and their use should also be avoided on such areas. It seems

clear that trees of virtually any species may be killed by F.annosus on the calcareous soils of Thetford, though losses caused in some species in the first few years after planting may be low. On the acid soils losses caused by F.annosus will be low.

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DEVELOPMENT OF DISEASE AND DYNAMIC OF PINE DIEBACK CAUSED BY FOMES ANNOSUS (Fr.) Cooke DURING 1971 AND 1972 AT DELIBLATO SAND

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INTRODUCTION

According to its geographical position, orographic features, specific climate and edaphic conditions, Deliblato Sand represents one of the most interesting object for biological investigations. In not to distant past, this region (36.568 ha) was covered by drifting sand, and in that time it was called "European Sahara". Deflation of sand was stopped by persistent man's efforts and today entire area is covered by vegetation. About 14.476 ha are forested and 4.8oo ha of it, are artificially established pine forests of the first and second rotation.

On the beginning of May 1969, at the locality Kremenjak, compartment 397, section "g", for the first time unexpected dieback of Scotch pine in this region was reported. Disease evolution and dieback of trees had acute character. Carpophores of Fomes annosus were found at the end of May and on the beginning of June of the same year when observation was made, at the ground level on trees at the final stage of disease development (M a r i n k o v i ć, P., 1972).

For a time being, disease is confined to acotch pine plantation in the above mentioned locality. It should be also emphisized, that forests and plantations of pine species at Deliblato Sand are isolated form autochthonous pine stands. The closest pine stands are located on southern slopes of Carpathian Mountains in Romania.

The objective of this report is to give a picture of condition of studied pine plantation, disease evolution, tendency of disease spread and intensity of dieback.

MATERIALS AND METHODS

In the above mentioned compartment 397, section "g", three sample plots, of about 1 ha in size, were selected. The basic principle for selection of these sample plots were configuration of the area (microdepressions) and the degree of infection by Fomes annosus.

Scotch pine plantation was established naturally on the area which have been clear cut. Trees in plantation are between 8 and 12 years old, with stocking varying from one part to the other. There are some dispersed Austrian pine and juniper trees. Soil is sandy, of recent genetic origin, partly cultivated. Present plantation represents second pine rotation. Total of 2.828 trees were talied, out of which 1.o32 trees are in sample plot N^{o} I; 1.129 trees on sample plots II; and 66o trees on sample plot III.

Every tree is tagged, described and thoroughly examined at regular monthly intervals during 1971 and 1972. This clinical procedure, of each tree, had as a goal to determine the time of the fungus activity, the way of disease spread, the stages in disease development and finally the dynamic of dieback of diseased trees. Each tree has its own file card where all changes have been entered. Died off trees have been cut down, stumps treated with pentachlorophenate, borax and urea on the purpose to be frustrated development of Fomes annosus carpophores.

Laboratory analysis was carried out on the purpose to be identified mycoflora inhabiting stumps, just before and after treatment procedure of stumps, in unregular time intervals.

Activity of fructification and dispersion of basidiospores of Fomes annosus at the same locality was observed.

RESULTS

Results which were derived in respect to disease evolution, spread of infection and intensity of dieback of diseased trees, are given in tables 1 and 2.

Table 1.-- Spreading of disease during 1971 and 1972.

No of Field	Date of Inspection	Number of Trees	Sound Trees		Diseased Trees	
			Number	%	Number	%
I	23.X.1971	1.o32	695	67,35	337	32,65
	23.X.1972		542	53,oo	49o	47,oo
II	25.X.1971	1.129	9o9	8o,51	22o	19,49
	25.X.1972		822	72,oo	3o7	28,oo
III	27.X.1971	667	38o	56,97	287	43,o3
	27.X.1972		284	42,oo	383	58,oo

In this table are given summarized results attained during the final inspection in 1971 and 1972. At the end of the first observation year 337, 22o and 278 trees with obvious signs of disease were evidented on I, II, and III sample area respectively. The heaviest attack was recorded for plot III (43 %), then for plot I (32,65 %) and the least for plot II (19,49 %).

During 1972, further intensive spread of infection was observed. The symptoms of disease were found in sample plot I on additional 153 trees, in sample plot II on 87 and in sample plot III 96, what together with diseased trees from 1971 comes up to: 49o, 3o7 and 383 diseased trees, respectively. The most intensive spread of infection was found to be for plot III (15 %), then for plot I (14,53 %) and the least for plot II (8,5 %).

Table 2. Develpment of Disease caused by Fomes annosus during 1971 and 1972.

No of Field	Date of Inspection	Number of Diseased Trees: Trees with Carpophore w/o chlorosis	Chlorotic Trees: Carp. F. annosus yes	Chlorotic Trees: Carp. F. annosus no	Dry Trees: Carp. F. annosus yes	Dry Trees: Carp. F. annosus no	Total of Diseased Trees
I	1971	63	28	125	73	48	337
	1972	75	24	1o8	148	135	49o
II	1971	8o	26	35	55	24	22o
	1972	61	37	58	1o7	44	3o7
III	1971	88	37	23	93	56	287
	1972	8o	32	42	144	85	383

In table 2 are given numerical relationships between diseased trees for different stages of disease development. Between initial symptoms of disease, which were manifested by slighty chlorotic needles, and die off of attacked trees, there are a few clearly defined transitive stages in development of disease. Chlorosis of needles becomes more evident and diseased trees at the end die off. In all stages of development of disease, on trees at the ground level, comes to formation of carpophores of Fomes annosus. In the column "Chlorotic trees", as a rule, number of trees with carpophores is smaller than number of trees without carpophores while in the column "Dry trees" that number is always much higher.

In column "Trees with Carpophore without chlorisis" number of diseased trees is relatively high. Such trees usually live slightly longer than chlorotic trees. Secundary symptoms of disease on these trees are slightly shorter needles and shoots.

Numerical values, in all columns during the year are changing, since at the end all infected trees eventually die off.

Observation of other trees with stated symptoms, formation of carpophores and dieback of trees, was made during entire period of inspection (March October). The intensive dieback of infected trees and the most prolific formation of carpophores was recorded during the period May-June (M a r i n k o v i ć, P., with collaborators, Annual Report for 1971).

DISCUSSION

Second rotation Scotch pine stands, between 8 - 12 years old, exhibited very high susceptibility to F. annosus at Deliblato Sand. After mature pine forest has been clear cut, it was replaced by stands established by natural regeneration. Remainings of cut trees are still visible, but we have not found infection by Fomes annosus.

K u h l m a n and R o s s (197o), however, emphisized that natural regeneration of seven pine species, Scotch pine was one of them, exhibited little mortality. The presence of natural regeneration in old centers of Fomes annosus infection, is not connected with the rapid depletion of the fungus, although pine stumps in the Southeast decompose very fast that after 8 years only fragments of bark could be found.

Considering this, in respect to these facts, high incidence of pine dieback at Deliblato Sand appears to be realy very interesting. It should be emphisized, however, that these natural regeneration stands have not been treated by any silvicultural measures, like thinning or pruning.

Regarding the origin of infection, it could be possible that it is from old stumps.

CONCLUSIONS

In natural regeneration on Deliblato Sand, Scotsh pine exhibited high susceptibility to F. Annosus.

Development of disease caused by F. annosus is followed by secundary symptoms which are manifested through different degree of chlorosis or occurence of shorter needles and shoots.

The final stage of disease developmet in both cases is die off of trees.

Carpophores of Fomes annosus are forming in all stages of disease development, but the most abundant formation of the same was observed during the stage of die off.

The most of dying off trees were recorded during the period May - June.

There is a tendency of further rapid spread of infection.

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DISCUSSION OF

REGENERATION OF INFESTED SITES

A. Yde-Andersen, Chairman

Pratt: Presented paper entitled "Fomes annosus butt rot. Losses in a young second rotation stand of Sitka spruce."

Driver: Are you getting any mortality in addition to your butt rot?

Pratt: No.

Driver: How about root decay?

Pratt: Yes.

Driver: How about windthrow? Early windthrow?

Pratt: Yes, this is quite a serious problem.

Driver: These symptoms are almost identical with our symptoms on western hemlock. We are finding a high incidence of butt rot in young stands of western hemlock on sites that are being regenerated (that is, they previously were natural western hemlock). We believe infection is coming from the previous stand. Sitka spruce is passing out of the commercial picture in the Pacific Northwest.

Pratt: Windthrow isn't something we've paid a lot of attention to but in terms of economic loss, I think it may be as important as plant failure (loss) on many sites. Another point is that many sites we are working on are former agricultural sites. They may have been in forest for 100 years but prior to that these were agriculture.

Driver: Our sites in the Pacific Northwest have always been forested with western hemlock.

Cowling: How much growth do you get with Scots pine in comparison with Sitka spruce on a similar site?

Pratt: Very variable, but I would guess about half.

Cowling: What do you do with logs that look like these you have shown us?

Pratt: Well, this is a difficult problem. Most people do all sorts of things with them. By that I mean when you are doing an economic evaluation, it is almost impossible to find one person who is doing the same thing as the person next door. In general, you can say that the section with bad decay is cut off and left in the forest as waste. The rest, depending on seasonal factors and what "old Joe" is thinking, is cut, taken to the mill, and is found quite acceptable. The estimates we make are all overestimates; the natural losses that occur are probably very much smaller.

Cowling: If you are aiming at the pulpwood market, then you might as well take it all. But I gather you are not selling at all for pulpwood?

Pratt: Well, we sell for pulpwood in the small (age) sizes, but some of our pulpwood people will not accept rot in their pulp mill. Depending on the type of pulp they are producing. I suppose they are not use to rot.

Cowling: Well, I think it is very clear that it makes good pulp.

Pratt: By the sulfate method process?

Cowling: By almost any process.

Yde-Andersen: It would be good to mention Bjorkman's work of 20 years ago that indicated pulpwood factories could use decayed wood and the yield would be up to 80% of sound wood.

Redfern: Rot in British forests is the exception rather than the rule so that if a consignment with stain or decay is taken to the mill, it is automatically rejected because it is so unusual. If a large portion of the timber were decayed, then I think we could persuade the companies to take it. (In the present situation this is not so, and it is rejected out of line.)

Dimitri: In Germany, 10 years ago decayed wood cut 3-4 meters and might affect 50% of the price of normal wood. But at the present time you can sell it. In 1972 we had a hurricane and got 50 million cubic meters of wood from windthrow. Twenty percent was lost.

Sylvestre: How did you select the trees to sample?

Pratt: We tried to choose the trees statisticians call an "ecological envelope" which is an area with ecological factors roughly the same--same soil type, etc. We also chose an area with Fomes because we were

not trying to do a survey of general Fomes development. We wanted to find out what is happening in the worst stands.

Sylvestre: Why did you sample at the base of the stump rather than at breast height?

Pratt: Because we were interested in determining loss potential and would have missed a lot at breast height.

Yde-Andersen: I realize you don't have all the previous history of the stand but do you know if the source of the infection is old infected stumps from a previous rotation, stumps from infected trees, or stumps from healthy trees that were infected by airborne spores? I think this is an important point because if the latter, then you know what to do, but if it is previously infected trees or stumps from earlier rotations, then it is much more difficult.

Greig: We have indications that it is infection of stumps at the time of clear felling that is important. Stump protection at the time of clear felling should control the situation.

Presented paper entitled "Fomes annosus: Mortality rates in young trees underplanted among pine."

Krebill: How do you dispose of your stumps after extracting them from the ground?

Greig: We leave them stacked in rows across the field. They decompose after 10-12 years. Some people have tried to burn them. Removal from the site is very expensive and we can't really afford to do any more than take them out of the ground.

Yde-Andersen: The stumps comprise 15% of the volume of the stem and in Sweden researchers are trying to utilize this stump wood for pulpwood on an experimental basis. It seems to be as good as stem wood.

Greig: We are interested in this in Britain and are following your work.

Courtois: Is the nutritive level of the soil correlated with growth of mortality?

Greig: I think the spread of the disease in the root system is different but whether this is nutritional differences or the differences in root surface microflora I am not certain.

Rishbeth: There was a good correlation with soil pH in this area of England. This does not happen everywhere, but did in this part of England. I agree with Greig that it is not certain if nutrition has an influence but soil microflora does have an influence.

Parmeter: Even with stump removal you had 20% infection. Do you assume inoculum is from the small fragments of roots that are not pulled out?

Greig: Yes. That first experiment was removal by winching which was crude and left a lot of roots. We traced roots and found contact in some cases with root pieces the size of your finger which had been broken off and left in the ground.

Parmeter: You mentioned the delayed planting had not worked. Wouldn't stump removal and a 2-3 year delay eliminate the problem?

Greig: Perhaps by the time of the next conference I will be better able to answer that one. We have an experiment laid out with stump removal and delayed planting. It is too early to say anything though. One problem is mortality does not occur until contact is made which is 3-4 years under our conditions. Most damage occurs after 7-8 years.

Cobb: In your tables fir had less damage. You say in the paper that slower growth and less contact contributed to that. Do you think those are the only reasons?

Greig: In this particular experiment the firs were very slow growing. Certainly other factors contributed. Some firs appeared to be much more resistant. In inoculation experiments Sitka spruce roots were infected up to the main stem following dowel inoculations in the roots, whereas Grand fir roots were hardly infected.

Driver: In your Table 2 Douglas fir appears to be most susceptible. What is the recommendation as to the use of this species on such sites?

Greig: Now it wouldn't be used on these sites. One of the interesting features of Douglas fir is the early high mortality. This stand was 8 or 9 years old. In other stands at Thetford Chase mortality in Douglas fir declined considerably at age 10-12. It appears high losses occur early and then stabilization follows.

Yde-Andersen: In Denmark we have conducted similar experiments. With Douglas fir we have had similar high initial mortality. Mortality ceases after 20 years. Then we have a 10-year period of high windthrow losses. At age 30 we have little or no butt rot. It is a question of surviving until age 30.

Cowling: The evidence for stumps and large structural roots serving as inoculum for the fungus is strong. How much evidence is there for small roots serving as inoculum?

Greig: I have done some excavating and root tracing and the smallest inoculum was perhaps 2 mm. This was a small root from a large stump which infected a 3-year-old Scots pine. The root was small but perhaps the inoculum source was large.

Cowling: Maybe then it is an assumption that small roots contribute to inoculum. An assumption and not a fact?

Greig: It is an assumption. However, following stump removal we have traced seedling roots to contact with roots about 1 cm in diameter.

Dimitri: The diameter must be above 1 cm to cause infection. If less than this diameter, the Fomes dies out.

Morrison: Do you foresee having to continue removing stumps on severely infested stands to keep your inoculum level low enough?

Greig: We hope not. The losses are important at ages 4-7 years, and then the disease stabilizes and very few trees die subsequently.

Morrison: How many trees would be infected when the disease stabilizes while the trees mature?

Greig: In the stand where we lost 25% it appears to have stabilized. Redfern was interested in determining if second generation trees that were killed were sources of infection for adjacent trees. This did not seem to occur. Subsequently, trees should not be infected if stump treatments are applied when thinnings are carried out. We are checking this experiment annually to determine if mortality decreases with age. Right now mortality has stabilized.

Rishbeth: Forestry Commission experience suggests that centers do stabilize with age even with large stumps and larger centers.

Hodges: Similarly in the Southeastern United States disease centers stabilize after 8-10 years with no further loss.

Shain: What is the explanation for this stabilization?

Skelly: When speaking of an infection center dying out, are you speaking of no more mortality or no new infections?

Shain: The term is commonly used. I believe it denotes no more dying in this infection center. More roots could be infected.

Skelly: I suspect there are many infected roots particularly on low hazard sites.

Shain: The fungus is still active?

Skelly: Yes, active but not killing trees. If initial infection is very low, the stumps producing the conks would stop producing after 5 years and the spore level would be reduced.

Hodges: I'm not sure that's what occurs in the Southeastern United States. At the end of 5 years, stumps are not active in spread of the disease whereas freshly killed trees are. Theoretically, the disease centers should continue to expand from tree to tree through root contact. Because more trees are involved as the center expands, the center should become more active. This does not happen after 10-year dying stops.

Myren: Is it possible stabilization is due to decomposition of the stumps? In Ontario I haven't noted any stabilization and stumps stay at least 10-15 years.

Hodges: I doubt that it is stump removal since the surrounding trees as they die are the inoculum source for further spread.

Myren: In selective thinnings wouldn't there be less chance for contacts other than with stumps? Don't your roots tend to go down as you go away from the tree?

Hodges: No, this is not the case in the Southeastern United States. On sandy sites the roots are only 4-6 cm deep and go 10-15 meters from the tree. Lack of root contact should not be the problem.

Yde-Andersen: Comments regarding the inability of small stumps to transmit the disease appear hastily considered. I believe these small stumps have spread the disease before they decompose.

Srago: There is no evidence of spread from pre-commercial thinning of ponderosa pine in the Western United States. Heat kills out the stump-surface infections in June, July, and August. On the other hand, the oldest infection center we have been watching is 33 years old and is still spreading at the margin. In the center where the oldest stump is located, young trees have started to be established with no evidence of mortality.

RESISTENZFORSCHUNG BEI DER FICHTE GEGENÜBER DEM FOMES ANNOSUS

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Die Untersuchungen über das Auftreten und die Beseitigung der vom Fomes annosus verursachten Schäden nehmen einen breiten Rahmen in der internationalen forstlichen Forschungsarbeit ein. Die nahezu 600 im vergangenen Jahrzehnt zu diesem Problem veröffentlichten wissenschaftlichen Arbeiten (HODGES et al., 1971) weisen eindeutig auf eine zunehmende Tendenz der Forschungsarbeiten hin. Nach wie vor sind aber die genetischen Fragen beim Erreger und bei den Wirtspflanzen bisher nur sehr unzureichend untersucht worden.

Um die erblich bedingte Widerstandsfähigkeit der Arten, Herkünfte und Klone der Gattung Picea ermitteln zu können, werden in der Hessischen Forstlichen Versuchsanstalt seit vier Jahren umfangreiche Untersuchungen durchgeführt. Durch zahlreiche Infektionsversuche an Fichtenwurzeln mit verschiedenem Alter im Labor und unter natürlichen Bedingungen im Walde, sollte zunächst die Frage geklärt werden, ob wir den Schwerpunkt der Untersuchungen auf die Ermittlung der Infektionsresistenz oder aber der Ausbreitungsresistenz legen sollten.

Die Ergebnisse unserer bereits veröffentlichten Arbeiten (DIMITRI, 1969 a, b; DIMITRI u. FRÖHLICH, 1972) haben uns überzeugt, daß für die Mehrheit der Fichtenbestände, die auf altem Waldboden stocken, die Ausbreitungsresistenz die wichtigere Rolle spielt. Auch die Infektionsresistenz wird zwar untersucht, aber schwerpunktmäßig arbeiten wir an der Ermittlung der erblich bedingten

Unterschiede in der Ausbreitungsresistenz. Hierüber soll nachstehend kurz berichtet werden.

MATERIAL UND METHODE

Da die Reaktionsweise und die Reaktionsnorm in unterschiedlichem Alter untersucht werden sollte, wurden 1- bis 80jährige Fichten als Untersuchungsmaterial verwendet. Das jüngere Material (bis 5jährig) wurde im Institut in Mitscherlich-Töpfen und die älteren Bäume unter jeweils vergleichbaren Bedingungen im Walde beimpft.
Die Anzahl der in den folgenden drei Altersgruppen beimpften und bisher untersuchten Fichten beträgt:

1 - 10jährig	600 Stück
11 - 24jährig	231 "
25 - 80jährig	200 "
insgesamt:	1.031 Stück

Als Erreger verwendeten wir stets Myzel und Konidien eines aus einer Basidiospore entstandenen Fomes annosus-Stammes (Stamm F_2).

In früheren Versuchen wurden aus den zu beimpfenden Bäumen im Wald unter halbsterilen Bedingungen Bohrspäne entnommen, in Konidiensuspension getaucht, in das Bohrloch zurückgeschoben und die Wundstelle mit Baumwachs verschlossen. Neuerdings werden in die Bohrlöcher andere, im Labor unter sterilen Bedingungen mit Fomes annosus-Myzel und Konidienträgern gut bewachsene Bohrspäne eingeschoben und gleichermaßen mit Baumwachs verschlossen. Hierdurch wird die Gefahr einer Verunreinigung durch Sekundärinfektion weitgehend verhindert und sichergestellt, daß in jede Wirtspflanze eine äquivalente Erregerpopulation gelangt ist. In die Stämmchen jüngerer Fichten wurden häufig durch eine Schnittwunde mit Fomes annosus-Myzel und Konidienträgern bewachsene Sägespäne als Infektionsmaterial eingeführt und die Impfstelle danach mit Baumwachs verschlossen.

Die Ermittlung des Infektionserfolges und der Myzelausbreitung in den Wirtspflanzen wurde nach unterschiedlich langer Einwirkungszeit durchgeführt. Von den abgeschnittenen Pflanzen bzw. gefällten Bäumen wurden zur Untersuchung Stammscheiben entnommen, numeriert und im feuchten Milieu bei Zimmertemperatur etwa 14 Tage lang aufbewahrt. Anschließend erfolgte die Untersuchung unter dem Binokular, wobei das Vorhandensein von Konidien-Rasen, Verfärbungszonen und sonstigen Veränderungen genau vermerkt waren.

ERGEBNISSE

Die Untersuchung der Ausbreitungsresistenz der Fichte gegenüber Fomes annosus infolge einer Infektion über die intakten Wurzeln, war wegen des äußerst geringen Infektionserfolges weder im

Labor noch unter natürlichen Bedingungen im Walde möglich. Gute Erfolge können jedoch erzielt werden, wenn die Infektion an der Stammbasis mit gutbewachsenem Infektionsmaterial erfolgt. Bei jungen Fichten konnten wir auch bei bester Methode nur am Ende der Vegetationszeit und während der Vegetationsruhe befriedigende Infektionserfolge erzielen. Je jünger die Wirtspflanzen sind, desto (1.) schwieriger war die Impfung, (2.) geringer der Erfolg einer künstlichen Infektion, (3.) geringer die Myzelausbreitung und (4.) desto schneller der Prozeß der Ausheilung. Junge Pflanzen zeigen wesentlich schneller und intensiver eine deutlich sichtbare Abwehrreaktion (Harzbildung, Holzverfärbung u.ä.m.), die die Entwicklung einer aussagefähigen Frühtestmethode erschweren könnte. Die Reaktionszone ist nicht bei jeder Klonfichte deutlich sichtbar und auch das Ausmaß der Beeinflussung kann von Pflanze zu Pflanze sehr unterschiedlich sein. In 7jährigen Klonfichten, in denen eine starke Reaktionszone entwickelt wurde (15-20 cm über der Infektionsstelle noch deutlich sichtbar), konnte Fomes annosus sieben Monate nach der Beimpfung überhaupt nicht oder nur sehr selten nachgewiesen werden. Daß das Holz der Reaktionszone pilzwidrige Stoffe enthält, konnte in verschiedenen Experimenten nachgewiesen werden (siehe Kliefoth).

Zwar ist auch bei 10-25jährigen Fichten ein Jahr nach einer künstlichen Beimpfung häufig eine deutliche Reaktionszone zu beobachten, Fomes annosus-Myzel läßt sich aber im Reifholz dieser Bäume trotzdem leicht nachweisen. Eine grau-grüne bis grau-braune Holzverfärbung geht über die Infektionsstelle wesentlich weiter hinauf, als die Myzelausbreitung (Tab.1). Die Harzeinlagerung ist hier bei weitem nicht so intensiv wie bei den jüngeren Pflanzen.

Die Differenzierung in der Myzelausbreitung kann innerhalb einer Teilpopulation auf Individualebene sehr groß sein (bis 1:12). Die Ergebnisse, die wir nach einjähriger Einwirkungszeit bei 17jährigen Klonfichten unter vergleichbaren Standortsbedingungen gewonnen haben, zeigen, daß diese großen Unterschiede in der Ausbreitungsresistenz nur teilweise auf genetische Faktoren zurückzuführen sind.

Tabelle 1: Ergebnisse künstlicher Fomes annosus-Infektionen bei 17jährigen Klonfichten nach einjähriger Einwirkungszeit

Klonbezeichnung	Durchmesser in 1,3 m cm	Infektionserfolg	Myzelausbreitung in % der Scheibenoberfläche (an der Bohrstelle)	Myzelausbreitung cm Höhe im Baum	Reaktionszone cm	Verharzungsgrad an der Bohrstelle	Variationsbreite cm
A	6,7	90	51	56,3	111	2,7	7-115
B	6,6	80	48	37,6	78	2,2	14-62
C	6,3	100	25	27,6	76	2,4	3-75
G	6,0	60	32	30,7	(60)	2,1	4-49
H	6,7	100	43	42,2	78	2,2	20-73
J	6,4	80	40	27,7	(38)	1,5	3-51
R	5,3	60	23	23,4	43	2,7	3-55

Zwischen den auf gleichem Standort wachsenden Klonen gibt es zwar sowohl im Infektionserfolg, als auch in der Myzelausbreitung beträchtliche Unterschiede, die Variationsbreite innerhalb der einzelnen Klone ist jedoch so groß, daß die Durchschnittswerte (aus jeweils 10 Fichten) gegeneinander nicht abgesichert werden konnten. Es ergaben sich aber trotzdem wichtige Hinweise. Bei etwa gleichem Durchmesser, Infektionserfolg und Verharzungsgrad bei der Impfstelle, konnten wesentliche Unterschiede in der Myzelausbreitung ermittelt werden (Klon A zu Klon C). Die Klone A und H verhalten sich in bezug auf die Myzelausbreitung trotz ausgeprägter Reaktionszone und guter Verharzung wesentlich ungünstiger als die Klone C, J und R. Auch bei gleicher Baumhöhe und gleichem Durchmesser in 1,3 m Höhe, kann die Myzelausbreitung bei verschiedenen Bäumen eines Klones sehr unterschiedlich sein. Worauf diese große Variationsbreite zurückzuführen ist, kann nicht eindeutig beantwortet werden. Sollten hierfür - wie vermutet - lokale Standortsbedingungen (insbesondere die Wasserversorgung des Bodens) maßgebend sein, so wurde in diesem Versuch die Bedeutung der genetischen Faktoren von den Umweltbedingungen bei weitem überlagert.

Auch in älteren Fichtenbeständen waren die Unterschiede in der Myzelausbreitung auf Individualebene wesentlich ausgeprägter als diejenigen zwischen den Teilpopulationen.

Die genetische Bedingtheit dieser Unterschiede in der Myzelausbreitung muß durch verschiedene Methoden (Holzabbau, Sporenkeimung und Myzelwachstum in Holzauszügen, biochemische Tests) möglichst unter konstanten, standardisierten Bedingungen und unter Verwendung von genetisch weitgehend bekannter Erregerpopulation (z.B. vegetative Nachkommenschaft einer Einsporkultur) ermittelt werden. Besondere Schwierigkeiten ergeben sich hierbei (1.) durch die geringe eigene antibiotische Aktivität von Fomes annosus (leichte Verunreinigung der Versuche durch Sekundärinfektionen), (2.) durch geringe Pathogenität des Fomes annosus (keine äußerlich deutlich sichtbaren Krankheitssymptome bei infizierten Pflanzen) und (3.) verhältnismäßig schnelles Ausheilvermögen bei jungen Pflanzen (Probleme beim Frühtest).

Da die Resistenz der Wirtspflanze nur in Relation zur Virulenz des Erregers gesehen werden kann, wird bei unseren Untersuchungen auch die parasitische Aktivität verschiedener Fomes annosus-Stämme unter natürlichen Bedingungen untersucht. In dieser Hinsicht konnten bisher nur geringe Unterschiede festgestellt werden.

SUMMARY

During the last four years research on the resistance of different species, provenances and clones of the Genus Picea against Fomes annosus were undertaken at the Forest Research Institute of Hessen. It was the main objective of our investigations to define

the differences in the active and passive resistance against spreading of this disease as far as they are caused by the genetic constitution of the research material. Using the same pathogene and applying it by the same method in the laboratory to young plants and in the field to older trees considerable, and for forest management important differences could be found. The genetic dependence of resistance is being tested with various method. Our field experiments showed so far only minor difference of the virulence of different Fomes annosus races.

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SPORENKEIMUNG UND MYZELWACHSTUM IN HOLZAUSZÜGEN VERSCHIEDENER FICHTEN

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Die bisherigen Kenntnisse über die natürliche Widerstandsfähigkeit der Fichte (Picea abies Karst.) gegenüber dem Rotfäuleerreger Fomes annosus sind sehr lückenhaft. Da aber zu erwarten ist, daß die Ausnutzung der erblich bedingten Resistenz eine ausgezeichnete Möglichkeit zum Einschränken der wirtschaftlichen Schäden bietet, werden die hiermit zusammenhängenden Fragen in der Hessischen Forstlichen Versuchsanstalt seit längerer Zeit schwerpunktmäßig untersucht (s. DIMITRI).

Die Widerstandsfähigkeit des inaktiven Holzes stehender Bäume kann auf chemischen und/oder morphologischen Gegebenheiten beruhen. Die bekanntesten Stoffe einer auf chemischen Bestandteilen beruhenden Resistenz sind das Pinosylvin in der Gruppe der Stilbene, das Thujaplicin in der Gruppe der Tropolene, das Quercetin in der Gruppe der Flavonoide u.a. Es wird jedoch angenommen, daß im Holz der Picea sp. keine oder nur wenige auf Mikroorganismen toxisch wirkende Substanzen vorhanden sind (SCHEFFER u. COWLING, 1966; GIBBS, 1968).

Die Tatsachen jedoch, daß (1.) in älteren Fichtenbeständen in unmittelbarer Nachbarschaft befallener Bäume solche stehen, die nicht oder in sehr unterschiedlichem Grade befallen sind und (2.) wir bei unseren künstlichen Infektionsversuchen an Fichten mit verschiedenem Alter (1- bis 70jährig), mit der gleichen Methode und derselben Erregerpopulation sehr große Unterschiede in der Myzelausbreitung bekommen haben, deuten auf die Möglichkeit einer beträchtlichen Differenzierung in der erblichen Resistenz hin. Um diese Möglichkeit näher zu untersuchen, wurden mehrere Labor- und Freilandteste durchgeführt. Einer davon ist die Sporenkeimung und das Myzelwachstum in Holzauszügen verschiedener Fichten.

MATERIAL UND METHODE

Als Untersuchungsmaterial diente Fichtensplint und -reifholz, das von Bäumen mit verschiedenem Alter und aus unterschiedlicher Baumhöhe entnommen war. In vielen Fällen wurde das Holz der unter natürlichen Bedingungen im Walde durch künstliche Infektionen als Plus- (geringe Myzelausdehnung; hohe Resistenz) bzw. als Minusvariante (große Myzelausdehnung; keine Resistenz) benannte Fichten als Untersuchungsmaterial genommen. Bei diesen sind auch aus der häufig vorkommenden Reaktionsholz-Zone Auszüge getestet.

Aus dem Sägemehl dieses Materials wurden zunächst kalte und warme Wasserauszüge angefertigt. Für diese Untersuchungen erwiesen sich die erstgenannten als geeigneter, weshalb wir in weiteren Versuchen nur diese verwendet haben. Hierzu wurden etwa 2-3 g Sägespäne mit dreifacher Wassermenge verrührt und 16 Stunden lang bei Zimmertemperatur aufbewahrt. Unter leichtem Druck wurde die Flüssigkeit abgegossen, aus der wir dann durch einen sterilen Membranfilter (0,45 u Porendichte) Filtrate erhielten. Je ein Tropfen dieser Filtrate wurde unter halbsterilen Bedingungen nacheinander auf 3 Deckgläschen gebracht. In diese kam eine annähernd gleiche, mit einem Pinsel von mehreren Stellen einer 14tägigen Kultur entnommenen Konidienzahl. Die Dichte wurde so gewählt, daß in einem mikroskopischen Blickfeld bei 320facher Vergrößerung etwa 30 - 50 Sporen zu sehen waren. Bei jeder Versuchsreihe verwendeten wir natürlich Konidiosporen einer Kultur. Das Deckgläschen wurde dann auf einen Hohlschliff-Objektträger gelegt, so daß ein Hängetropfen über einem feuchten Raum entstand, der durch kapillar eingesaugten Wasserrand abgedichtet war. Bei Kontrollpräparaten wurde eine 1%ige Malzinlösung als Nährmedium verwendet. Die auf diese Weise vorbereiteten Objektträger wurden auf Glasstäben in einem Plastikbehälter, an dessen Boden sich ein feuchtes Filterpapier befand, bei Zimmertemperatur aufbewahrt.

Die Untersuchung der Sporenkeimung und des Myzelwachstums erfolgte unter einem leistungsfähigen Photomikroskop bei 320facher Vergrößerung nach 24 Stunden. In einigen Fällen wurde diese Zeitspanne durch zwischenzeitliche Untersuchungen nach 6, 8 und 10 Stunden unterbrochen bzw. auf 48 und 72 Stunden verlängert. Bei jedem der drei Präparate einer Probe betrachteten wir repräsentativ 3 bis 5 Gesichtsfelder, wobei die gekeimten und nichtgekeimten Sporen ausgezählt und bei den erstgenannten die Hyphenlänge jeweils gemessen wurde. Zahlreiche, in unterschiedlichen Zeitabständen angefertigte Photos sollten die charakteristischen Entwicklungsphasen verdeutlichen.

ERGEBNISSE

Die nach der oben beschriebenen Methode hergestellten Präparate sind - von einzelnen Ausnahmefällen abgesehen - sauber geblieben.
Vergleicht man die durch diese Methode gewonnenen Ergebnisse mit der Myzelausbreitung in stehenden Bäumen, so ist in Tab. 1 eine gute Übereinstimmung zu sehen.

Tab. 1: Sporenkeimung und Myzelwachstum in Holzauszügen aus Fichten, in denen derselbe Erreger im Walde eine große (Minusvariante), beziehungsweise eine geringe Ausdehnung (Plusvariante) erreicht hatte .

Baum-Nr.	Alter	Myzelausdehnung im Baum in cm	Sporenkeimung in %		Myzelwachstum in µ	
			Vers.1 16 Std.	Vers.2 24 Std.	Vers.1 16 Std.	Vers.2 24 Std.
Ga 9	40	310	84	78	57	151
Ga 11	40	5	57	52	32	83
Hi 1	33	214	73	72	48	120
Hi 9	33	64	63	57	39	105
Ok 2	61	252	77	72	52	187
Ok 16	61	81	76	54	44	139

Sowohl die Sporenkeimung als auch das Myzelwachstum wird in Holzauszügen aus Plusvarianten (Baum-Nr. Ga 11, Hi 9 und Ok 16) wesentlich gehemmt. Die Unterschiede bei den Beobachtungen des Myzelwachstums konnten durch den F-Test hochsignifikant abgesichert werden. Diese Beziehungen konnten auch bei 17jährigen Klonfichten, die nach der bei DIMITRI näher beschriebenen Methode beimpft und nach einem Jahr untersucht waren, gefunden werden (Tab. 2).

Tab. 2: Sporenkeimung und Myzelwachstum in Holzauszügen aus Klonfichten, in denen derselbe Erreger im Walde eine große, beziehungsweise eine geringe Ausdehnung erreicht hatte.

Nr.	Baum-Alter	Myzelausdehnung im Baum in cm	Sporenkeimung in %	Myzelwachstum in µ
R 4,9 und 10	17	18	71	372
H 5,7 und 10	17	39	83	863

Betrachtet man die Wirkung der aus verschiedenen Jahrring-Gruppen entnommenen Holzauszüge auf das Myzelwachstum, so können in einem Querschnitt drei Zonen unterschieden werden: (1) Reifholz, (2) Splintholz und (3) die Übergangszone zwischen 1 und 2. In Abb. 1 sind bei den zwei untersuchten 115 Jahre alten Fichten die drei Zonen deutlich sichtbar, in der Intensität der Hemmwirkung unterscheiden sie sich jedoch grundsätzlich. Etwa bis zum 25. Jahrring die trockene Reifholzzone und ab dem 82.Jahrring die Splintholzzone. Dazwischen befindet sich die größte Zone, in der - nach unseren Beobachtungen - sowohl unter natürlichen Bedingungen im Walde als auch in den Holzauszügen im Labor das Fomes annosus -Myzel am besten wächst.

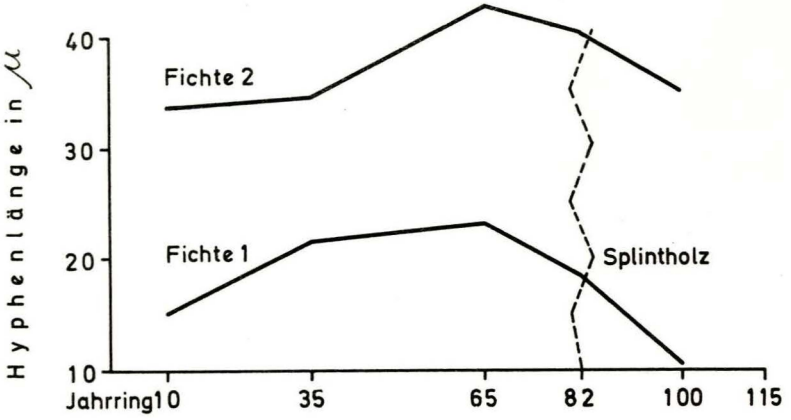

Abb. 1: Myzelwachstum in Holzauszügen aus der Reifholz-, Splintholz- und der Übergangszone zweier 115jähriger Fichten.

Will man die Unterschiede verschiedener Fichten-Individuen im Hinblick auf die mögliche Ausbreitungsresistenz untersuchen, so sollte die Probeentnahme stets aus der gleichen Zone erfolgen. Hierfür eignet sich die Übergangszone am besten, da hier das Myzel gut wächst und die Differenzierung am deutlichsten hervortritt.

Wir konnten die bei SCHEFFER und COWLING (1966) beschriebenen Befunde auch bei Fichte gegenüber dem Fomes annosus bestätigen, wonach die Hemmwirkung in einem Baum vom Stammfuß nach oben zu und in horizontaler Ebene von außen nach innen abnimmt.

Aus den nachstehenden Zahlen geht die Bedeutung der Ausbildung einer Reaktionszone in der Wirtspflanze nach Beimpfung mit Fomes annosus hervor:

	Sporenkeimung in %		Myzelwachstum in u		Bemerkung
	a	b	a	b	
Klon 4, Baum-Nr. 10 [1]	72,0	50,0	92,5	84,9	ohne Reaktionsholz
Klon 4, Baum-Nr. 16 [1]	49,0	41,0	51,0	89,0	mit Reaktionsholz

[1] 8jährige Fichten
a = Probeentnahme in der Nähe der Impfstelle
b = Probeentnahme in 20 cm über der Impfstelle

Sowohl die Sporenkeimung als auch das Myzelwachstum war in Holzauszügen bei Proben, die 20 cm über der Impfstelle (von Fomes annosus)unbeeinflußte Zone!) entnommen waren, nahezu gleich. Holzauszüge aus der Reaktionszone (grau-violette bis braune Holzverfärbung) haben die Sporenkeimung aber auch das Myzelwachstum deutlich gehemmt. Dies könnte ein Grund dafür sein, daß (1.) der Infektionserfolg und die Myzelausbreitung unter natürlichen Bedingungen im Wald bei Bäumen, die nach der Beimpfung eine Reaktionszone ausbilden, wesentlich geringer ist sowie (2.) die Verdrängung des Erregers (Ausheilung) häufiger und schneller bei solchen Bäumen vorkommt (s.DIMITRI).

SUMMARY

The objective of the research work described above was to confirm differences of the resistance against spreading of Fomes annosus in the host between individual trees of Picea abies found in field tests by investigating germination of spores and growth of mycelium in wood extracts in the laboratory. The results obtained indicate that wood of trees identified in the field to have relatively high spread-resistance against Fomes annosus contains apparently substances which retard germination of spores as well as growth of mycelium. Nature and amount of these substances have not been determined by this research project. However, there exist apparently variations between individual trees of P.abies caused by genetic or environmental factors. It is not possible to define definitely differences of resistance between individual trees against Fomes annosus by the method outlined above, but is a quick and reliable test easy to handle.

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RELATIVE SUSCEPTIBILITY OF SLASH, LOBLOLLY AND LONGLEAF PINES TO INFECTION BY *FOMES ANNOSUS*

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Fomes annosus attacks a large number of species in most coniferous genera with only a few species having been reported to have some degree of resistance. In the southern United States, the fungus is present on all native species of pines and causes especially heavy damage in thinned plantations of loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm. var *elliottii*) pines (7). Little is known about the relative susceptibility of longleaf (*P. palustris* Mill.), another important species of southern pine. Siggers (8) observed several longleaf trees dying in a 9-year-old plantation in Louisiana while Kuhlman and Ross (5) reported minor losses in a 4-year-old planting on a site infested with *F. annosus*. Infection centers of *F. annosus* have been observed in a relatively large number of longleaf pine plantations by the writer; however, it appeared that losses were less than in plantations of slash and loblolly on similar sites, thinned for similar lengths of time, even if large numbers of infected stumps were present. Kuhlman (3) also reported longleaf pine seedlings to be somewhat more resistant to *F. annosus* in greenhouse inoculation tests.

This study was designed to determine the relative susceptibility of longleaf, loblolly and slash pines. Some preliminary data were reported earlier (1).

MATERIALS AND METHODS

The study was established in January 1968 in unthinned plantations of 13-year-old slash, loblolly and longleaf pines on the Savannah River Project (Atomic Energy Commission) near Aiken, South Carolina. Spacing in each plantation was 1.8 x 2.5 m. The longleaf and loblolly pine plantations were contiguous while the slash pine plantation was about 400 m away. The plantations were located in the coastal sandhills, with an A horizon from 50-110 cm deep; these are high hazard sites (6).

Susceptibility to F. annosus was assessed by (a) determining the rate of fungus growth in inoculated roots and (b) measuring the rate of spread of the disease from artificially established infection centers. In the first method, two roots, 2-5 cm in diameter, on each of 27-28 trees of each species were inoculated by drilling a 12-mm diameter hole in the root at least 45 cm from the root collar to a depth slightly less than root diameter. An infested piece of pine branch, approximately 12 x 18 mm, on which F. annosus had been growing for 3 months, was inserted in the hole and immediately covered with molten wax. The root was then covered with soil. Six months later, the roots were severed at the root collar and returned to the laboratory. They were washed, cut into 30-cm sections, dipped in a 0.05% solution of sodium hypochlorite for 2 to 3 min, and each section split lengthwise. Isolations were made at 2.5-cm intervals for at least 45 cm proximally and distally to the point of inoculation with a Swedish increment hammer. Two cores were removed at each interval and plated on a F. annosus medium (4). Extent and location of resin exudation on the phloem or infiltration of the xylem were noted and compared with the isolation data.

The rate of spread of F. annosus in the three plantations was measured from artificially established infection centers. Ten centers were established in each plantation by cutting 4 adjacent trees, 2 in each of 2 rows, and inoculating the stumps with F. annosus. The trees were severed about 20 cm above the ground and the stumps then severed again 8-10 cm above the ground. The second surface was then sprayed with a conidial suspension of F. annosus. The short section of stem resulting from the second cut was then placed on the stump to intercept possible antagonistic microorganisms. The stumps were sampled 2-5 cm below the surface with an increment borer 3 months later and the cores plated on the F. annosus medium to determine the number of stumps infected. Final data on spread of the fungus to adjacent trees were taken in December 1972, 5 years after inoculation.

Maximum spread of the fungus was measured from a point in the center of the four inoculated stumps (plot center) to the most distant dead or infected tree in any direction. Spread across the rows was measured from the plot center on a line perpendicular to the rows to the furthest row in which a dead or infected tree was found. Spread down the row was measured from the plot center along a line parallel to the rows to the furthest dead or infected tree in any row. Dead trees were considered to have died from F. annosus if a conk or typical root decay was present or if F. annosus was isolated from the roots. Living trees were considered to be infected if one or more major roots were infected.

RESULTS AND DISCUSSION

The percentage infection and average growth of the fungus proximal to the point of inoculation for the three species are shown in Table 1. These averages do not include roots from which F. annosus was not isolated. Average growth of the fungus distal to the point of inoculation was not computed since the distal portion of many of the roots died and the fungus grew to distances greater than were sampled, in some cases more than 1 m.

Table 1.--Percent infection and average growth of Fomes annosus in inoculated roots of loblolly, slash and longleaf pines

Species	Number of roots	Percent roots[a] with F. annosus	Average growth rate (cm)[b]
Longleaf	55	11	5.8
Loblolly	53	86	16.0
Slash	54	70	21.0

[a]Roots with F. annosus proximal to point of inoculation.
[b]Growth rate computed only in portion of roots proximal to point of inoculation and only for infected roots.

The percentage of roots infected and the average proximal rate of growth was similar in loblolly and slash pines. The maximum growth rate recorded was 65 cm in one loblolly pine root. In slash pine the maximum growth recorded was 32.5 cm. In loblolly pine the fungus could usually be isolated at all points between the point of inoculation and its maximum point of growth. In slash roots, the fungus was often isolated only from an area near its maximum point of growth.

Percentage infection and rate of growth in longleaf pine roots were less than in the other two species. The fungus was recovered from only 6 of the 55 roots inoculated and had progressed 12.5 cm from the point of inoculation in 2 roots and 2.5 cm in the other 4 infected roots.

Copious resin exudation and infiltration was noted on slash and loblolly pine roots, being somewhat greater in the former species. Less resin infiltration and exudation was noted on longleaf roots. On loblolly roots the area with resin exudation and xylem infiltration more or less corresponded to that where the fungus was isolated but in slash pine roots copious resin exudation often occurred at points where no resin infiltration was present and the fungus was not isolated from the xylem. This suggests that F. annosus may be growing in the phloem and eliciting response from the root in the form of resin exudation without actually penetrating the root xylem.

The average number of loblolly pine trees killed by or infected with F. annosus and the distance of spread from the artificially established infection centers was significantly greater than similar values for longleaf pine (Table 2). The average number of dead and infected trees and the average rates of spread was less in slash pine than in loblolly but these differences were not significant except for spread across the rows. Longleaf pine also had significantly less trees infected or dead than slash but the differences in the rates of spread were not significant. The differences in maximum and down row spread between slash and longleaf were nearly significant, however.

The reason for absence of any spread in slash plot 10 is not known. When the stumps were checked 3 months following inoculation, F. annosus was isolated from all four stumps in the plot. The roots of the stumps and the adjacent trees were excavated and while some contacts were observed, no infection was found in any tree roots. The stump roots were too badly decomposed to determine if F. annosus had been present. Since some spread occurred in 9 of 10 of the slash plots, lack of susceptibility cannot be the explanation for lack of spread. It is more likely that adequate contacts between infected stump roots and the adjacent tree roots did not occur. If this plot is not included in the computations, then longleaf pine would be rated significantly less susceptible than loblolly and slash in all parameters measured except for across row spread in slash pine.

The only significant difference between loblolly and slash pine was the rate of spread across rows. In loblolly pine spread down and across rows was approximately the same. In slash pine, dead or infected trees were seldom found more than one row beyond the row containing the inoculated stumps, while down row spread occurred up to four trees away from the nearest stump. Since down row spread in the two species was approximately equal, the differences in cross row spread is probably due to differences in rooting patterns.

The maximum rates of spread in loblolly and slash pines average 1.3 and 1.1 m per year and are similar to the average rate reported by Hodges (2) in natural infection centers in an unthinned slash pine plantation on a similar soil type. This is probably representative of the potential rate of spread of F. annosus in plantations of these two species on high hazard sites.

The data presented here represent the first report of spread of F. annosus from artificially established infection centers and describes a technique that can be utilized to study the influence of edaphic and other factors on the spread of F. annosus.

The results of both the root inoculation and the spread studies indicate that while longleaf pine is susceptible to F. annosus, it is less so than slash and loblolly pine. This knowledge, added to the facts that longleaf pine is also more resistant to fusiform rust than loblolly and slash pines and has other more desirable traits should add further impetus to the effort to utilize longleaf pine on sites where the hazard from rust and annosus root rot is high.

Table 2.--Numbers of trees killed or infected after 5 years by *Fomes annosus* and spread of the fungus in artificially established infection centers in slash, loblolly and longleaf pines

Plot No.	No. dead and infected	Spread (m)		
		Maximum	Down row	Across row
		Loblolly		
1	14	9.5	6.7	6.5
2	9	3.9	3.9	2.6
3	13	6.5	6.5	5.9
4	9	5.4	3.6	4.0
5	18	7.8	4.1	7.8
6	15	6.3	6.1	5.6
7	15	7.7	7.6	5.5
8	12	6.3	6.2	3.7
9	15	6.4	5.3	6.2
10	11	6.6	6.0	6.3
Ave.	13.1	6.6	5.6	5.4
		Slash		
1	10	6.2	5.0	3.7
2	9	6.3	5.6	3.4
3	17	7.2	6.9	3.5
4	11	6.0	5.9	3.2
5	14	6.3	5.7	3.8
6	13	7.1	7.0	5.6
7	12	7.4	7.2	3.6
8	8	4.7	3.6	3.2
9	7	4.1	3.5	3.6
10	0	-	-	-
Ave.	10.1	5.5	5.0	3.4
		Longleaf		
1	8	4.1	4.0	2.7
2	5	4.0	3.9	0.6
3	8	4.6	4.3	2.0
4	9	4.3	3.9	4.2
5	6	3.2	3.1	2.5
6	8	4.1	4.0	2.9
7	3	3.6	3.2	3.1
8	5	4.1	2.4	4.0
9	6	3.9	2.6	2.4
10	9	5.0	4.8	5.0
Ave.	6.7	4.1	3.6	2.9
LSD-0.05 for ave.	3.1	1.5	1.4	1.4

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RESISTANCE OF PINUS SILVESTRIS TO THE ROOT - ROT

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Hitherto there have not been developed any effective means of checking up this dangerous disease. Thus far, all the suggested measures of the root-rot control in most cases have been resulting in the substitution of foliage species for the infected coniferous stands or there were recommended uneffective methods of a prophylatic character (thinnings). However, all these arrangements do not guarantee stand protection to the disease, therefore new ways must be sought as to its check-up, i.e., the study of tree resistance to the disease.

The resistance of individual pine specimens is referred to by A.M.Annudinov (1951). Such trees are endowed with a mighty, well-developed root-system, placed in deeper layers of the soil.

Likewise P.I.Klushnik (1962) deals with pine resistance in the hotbed of infection: individual trees remain healthy, while all the rest, being less resistant, have already withered.

The problems of the resistance may be also observed in I.J.Shemyakin's research (1963). The aspect of resis-

tant trees is as well marked by A.G.Chernykh (1966).

In the sphere of the resistance our investigations have already commenced in 1960, and hence it was found there are pines in the forests of Latvia offering a considerably heightened resistance to the root-rot infection (A.J.Lauska, 1961). Subsequently a more elaborate research of the symptoms, characterizing the resistance of indivi - dual pine specimens was carried out. To search for new ways of the root-rot control, basing on natural resistance of trees, was studied the resistance of pine and spruce to the artificial infection with fungus Fomitopsis annosus. Resulting from this experiment it was stated that the least possibility of pine infection was observed on peat soils, pine nurseries proving to be less resistant to the root-rot (A.J.Lauska, 1970).

Pines offering resistance differed from the ordinary specimens by their morphological indications and anatomical structure of the wood (A.J.Lauska, 1970).

In like manner many investigations abroad have been devoted to the problem of the resistance. Gibbs I.N. (1968) has accomplished a large-scale research concerning resinous qualities of the wood of the resistant trees against Fomitopsis annosus found in the specimens of coniferous species.

According to Jörgensen E. (1961) and Shain I. (1967) research, pinosilvin is of major importance as to the resistance of pine. The authors maintain the opinion that fenol combinations of this group are weighty in the resistance of the undergrowth.

Sinclair N.A. (1964) and Shain I. (1967) lay stress on the processes of accumulation of resinous substances playing the principal role in the resistance of trees.

In 1964 in Forest Experiment Station "Kalsnava" seedlings from selected individual specimens of the resistant pine were raised in the open air by means of the sowing method. Thereupon in Krievupe Forest Range of Riga Forest Enterprise experimental cultures were planted from the obtained seedlings in the so-called "isolation", created in the hotbed of infection. Four resistant pines A, B, C, D were used in the experiments:

A and B : growing in Krievupe Forest Range of Riga Enterprise,
C : -"- in Jumara Forest Range of Valmiera Enterprise,
D : -"- in Birzgale Forest Range of Jaunyelgava Enterprise.

A and B are characterized by the same site conditions used for raising of the experimental stands: Cladonia, Bonity III-IV, 40 years, 100% Pine. Reaction of the soil from pH 4,2 to pH 6,7. Middle height of

trees - 16,0 m in A, 17,0 m in B. Diameter at the breast height - 20,0 cm in A, 22,0 cm in B. Territorially these stands are situated in the western part of the republic on sand dunes.

Pine C is located in richer site conditions: Vaccinium, Bonity II, age of the stand - 45-50, middle height of trees - 20,5, diameter at the breast height - 28,0 cm. Reaction of the soil from pH 4,3 to pH 6,9. The stand is situated in the eastern part of the republic on a plain lowland.

Pine D is qualified by site conditions equal to the afore-mentioned: Vaccinium, Bonity II, age of the stand 47 - 50, middle height of trees - 19,5 m, diameter at the breast height - 28,0 cm. Reaction of the soil from pH 4,0 to pH6,5. The stand is found on a dry, plain locality in the central part of the republic.

The indices of the progress of growth and development of the experimental 7-year stands are presented in table 1 (the results of 1971). The difference between the control and experimental variants is essentially distinctive in all the cases, plausibility reaching 95%. The stands of the control variants are composed of healthy two-year-old seedlings drawn from the local nursery.

Table 1.

Variant	Height cm	Height increment, cm	Length of the needles, cm	Diameter at the root-neck, cm
A	104±6,09	25±2,48	5±0,29	2,3±0,14
Control	68±8,78	15±2,93	4±0,22	1,7±0,15
B	102±4,19	27±1,73	6±0,23	2,5±0,13
Control	77±7,90	21±2,50	4±0,33	1,5±0,14
C	110±5,75	31±1,30	7±0,28	2,8±0,15
Control	67±8,86	18±2,66	4,5±0,57	1,6±0,29
D	107±8,37	25±0,26	6±0,38	2,8±0,21
Control	62±2,57	11±1,47	5±0,15	1,7±0,43

Furthermore, survival of the trees raised from seeds of the resistant pines was high; yet the control variants in some cases had withered away by more than 50%. Survival of the stands may be seen in table 2.

Simultaneously with the formation of experimental cultures, inoculation of resistant pines was done, hence they have started to produce seeds.

Table 2.

Variant	Number of trees (%)	
	Growing	Dead
A	94,1	5,9
Control	62,5	37,5
B	100,0	-
Control	50,0	50,0
C	83,3	16,7
Control	43,5	56,5
D	82,4	17,6
Control	52,6	47,4

A considerably high survival was fixed in the clone of the local origin (A and B). The withered trees of the control variants had damaged roots due to the root - -rot.

The obtained information makes us believe the new course leading to the root-rot control will be far-reaching and perspective. These results, however, must be tested on a wider-scale experimental material.

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DISCUSSION OF PAPERS ON RESISTANCE AND RELATIVE SUSCEPTIBILITY

Richard Parmeter, Chairman

I. Discussion of papers by Dimitri and Klieforth

Klingstrom: Is *Trichoderma* also effective at lower temperatures?

Dimitri: We didn't examine the influence of *Trichoderma* on *F. annosus*.

Yde-Andersen: As far as I could understand, you are dealing with two sorts of resistance — resistance to infection and resistance to growth in sapwood. In your experiments, you wounded trees, therefore you have taken away the first type. You are dealing with small trees that do not yet have heartwood, and it is resistance in the sapwood that you are dealing with for the moment.

Dimitri: We have worked with trees 5-70 years old. With trees older than 20 years, we inoculated with a cork borer at 1.5 m. If we find differences among injured trees, we need to know where the differences are coming from. As I will show in Klieforth's paper, we grafted cuttings from test trees, and we will inoculate these under controlled conditions to find out if resistance in grafted cuttings gives the same results. If results with 1-4 year old grafts is the same, this means resistance should be genetically controlled.

Parmeter: You're using only a single clone of the fungus?

Dimitri: Yes.

Parmeter: Then you have no idea how they might react to another clone.

Dimitri: Yes. We examined different clones and found that our clone (F_2) infects rapidly and produces the most conidia. But we have little knowledge on virulence. When we infected the same trees with three clones, F_2 was always best. Like Mr. Roll-Hansen, we have been unable to find significant differences among clones.

Roll-Hansen: There are small differences.

Dimitri: Yes, but not significant differences.

Parmeter: In California we see *F. annosus* widely spread in *Abies concolor*, usually causing buttrot but in some places causing spreading centers of mortality. We've wondered if spreading mortality centers involved especially virulent clones in contrast to usual buttrot infections.

Dimitri: Since we know that a square centimeter of fruiting body can produce up to five million spores, we know that much genetic variability is possible.

Shain: Were you testing infected or uninfected seedlings?

Dimitri: Uninfected.

Shain: Was the bark removed before you made your extracts?

Dimitri: No. They were extracts of wood from infected trees — and always removed from the same height. We determined weight loss after six months by the soil block method. Then Mr. Klieforth took hot and cold water extracts.

Shain: This was after decay?

Dimitri: Yes. After decay.

Shain: Then you weren't looking for products from living tissues.

Dimitri: We took samples from decay, the reaction zone, and at 5-6 cm [from the decay].

Shain: What results did you get from the reaction zone on plus and minus trees?

Dimitri: Mr. Klieforth made these tests, but I don't have his results.

Parmeter: Was there a correlation among results from heartwood, inner sapwood, and outer sapwood?

Dimitri: Yes.

Roll-Hansen: If the seedlings had been stressed, the fungus might have killed them. In one of our experiments compared well-watered with stressed trees. We got nice results with stressed trees.

Dimitri: I agree, but how do you infect them? I tried to infect them through roots, but there were no symptoms, the fungus grew only

2mm, and after a year, it couldn't be isolated.

Courtois: Let me comment on two points. First, is it best to fight against infection [or against growth in the tree]? Our work shows that many conidia have only one nucleus and are homokaryons. What kinds of possibilities can result after anastomosis, plasmogamy, and karyogamy which change the characteristics of the fungus and its reaction to new environments?

Dimitri: I agree that we have to examine infection too. But we worked two years to find out if *F. annosus* could grow in healthy, undamaged roots, and you know the results. It couldn't, so resistance to growth in the wood must be important. It may be that the fungus can change and that variability can be produced in the laboratory. But our studies and the reports in the literature indicate that we can't find significant differences in field isolates.

Roll-Hansen: I think it might be possible to get mycelium from uninucleate conidia, and basidiospores are mostly uninucleate.

Dimitri: If we found that some trees resistant to F_2 are not so resistant to another clone, it could be that they would still be more resistant than would minus trees against this strain too. This is only a first step. We find that it is not so easy to propagate resistant trees, since only about 5% of the cuttings root.

Courtois: What was your object in breeding for resistance?

Dimitri: My object is to find at least 100-160 clones which are resistant genotypically and phenotypically. These trees might be propagated vegetatively or used for seed orchards.

Driver: When you determined the nuclear condition of spores, was this during maturation or after the spores had matured and dehisced?

Dimitri: We used mature spores.

Courtois: We stained mature spores and examined with a microscope.

II. Discussion of the paper by Hodges

Driver: It's important to examine spores at the time they're cut out, because uninucleate spores can become multinucleate as they mature.

_____: I did the same thing with loblolly once. Not only did I get

no infection, I got significant increase in the growth of loblolly.

Hodges: I can't explain that.

Sylvestre: Do you know anything about the chemical composition of the wood between trees?

Hodges: No. We haven't done that type of physiological work.

Dimitri: Are there differences in the roots between species?

Hodges: Yes. There was a difference in the amount of resin exudation and an inverse relationship between the amount of resin production and the infection. For instance, in slash pine there was tremendous resin infiltration and exudation into the soil. Whereas in longleaf pine, there was very little resin exudation.

Yde-Andersen: Is there heartwood in all species?

Hodges: At the age of the trees we used there is no heartwood — at least no discernible heartwood. But all three species form heartwood.

_____: Do you know to what extent roots have to be wounded before you can get infection?

Hodges: No. Even though I try to prevent wounding, I'm sure it occurs during digging. Even with that, I got no infection.

Pratt: Are there any major differences between the rooting habits of the three species?

Hodges: Not in the type of soil in which they were growing. All three produce spreading root systems and taproots.

Alexander: From your previous reports, how do you explain your observations that you get infection in a part of the root with no infection at either end?

Hodges: It's quite common under southern conditions to find infections at points away from any root contact. My explanation is that the fungus grows in the bark, and where there's a wound — or for unknown reasons — it causes infection. This is an interesting problem. From my work and from the literature, we find an average rate of spread of about 1.3 m per year, but the rate of growth through living roots is about 40 cm per year. One explanation is that the fungus grows faster through the bark than through the root, as Dr. Rishbeth suggested years ago.

Stambaugh: Don't you think that some of the inoculation failure through intact roots is related to food base?

Hodges: This is quite possible, but it's a difficult problem. We discussed earlier the question of how large a piece of root is necessary to cause infection. The point of root contact may be only a few square centimeters, but we may have a root three meters long

attached to a stump with a cubic meter of volume and all infected with *F. annosus*. What is the inoculum potential? Does the whole stump and root system take part? I think this makes a good problem.

Stambaugh: In some of our preliminary work with cedar, our results were similar to yours. But if we took the same sized inoculum segments and completely encircled the root, we did get some infection.

Hodges: How much?

Stambaugh: Enough to prompt us to want to do more work.

Rishbeth: I've been concerned with this problem for many years. I've worked mostly with light-textured soils, where we've had failures even where the fungus persisted in inoculum blocks. I've thought — and others have suggested it, too — that one of the factors preventing *F. annosus* from coming out of the inoculum block might be gradual desiccation. Recently one of my students enclosed inoculum blocks with moist clay or polyethylene after the blocks were firmly attached to roots. With this system, he got very effective transfers from relatively small inoculum.

Parmeter: Literature review suggests that *F. annosus* is more likely to spread from the stumps of some species than from others. Could the reduced rate of spread from longleaf pine be due to the fact that it is not as good a food base?

Hodges: No. I think longleaf pine may be a better base. Longleaf pine stumps have more heartwood, higher resin content, and greater persistence.

Johansson: Resistance may depend more on the quality than on the quantity of resins. There are differences in the quality of resins in the reaction zone of spruce, and there may be other components, since the reaction zone is very active against *F. annosus*.

Hodges: I'm sure there is work on the chemical composition of southern pines, but offhand I don't know what the differences are.

Cobb: Several years ago we did a study that showed that the quality — or at least the different components — of ponderosa pine resins had different effects on *F. annosus* and other fungi as well. When we tested volatiles collected in the spring from living phloem, spore germination and mycelial growth was completely inhibited. When the study was repeated in the fall of the year, we got only minor inhibition. We haven't followed up this work as yet.

Marx: There has been some work done on production of volatile compounds in response to mycorrhizal infection. These compounds, mainly terpenes and sesquiterpenes, are strongly inhibitory to *F. annosus*.

Cobb: In relation to that, we got inhibition when fungi were exposed to volatiles in the atmosphere, where some oxidation probably occurred. If tests were made in liquid culture, where presumably we didn't get volatilization, we found little effect. Dr. Zavarin and I have hypothesized that oxidized terpenes rather than non-oxidized terpenes may be the key.

THE EFFECT OF PHYTONCIDES, ROOT-AND LEAF EXTRACTS OF SOME PLANTS ON THE GROWTH OF FOMES ANNOSUS MYCELIUM IN CULTURE

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INTRODUCTION

The allelopathy means toxic effect of one plant on another one by means of external metabolic agents. Such compounds may be excreted by roots, washed from leaves or residue and also evaporated from various organs of plants. Some species of plants are known not to be able to germinate in the forest community because some substances washed away from bedding inhibit the germination and growth of germs. In South Caroline cherry-rooted oak excretes substances that oppress the germ growth of other species of plants under its crown (2).

The volatile resins of pine (Pinus ponderosa) inhibit the growth of the mycelium of the fungus Fomes annosus (1).

The presented and other data indicate that in the nature many different kinds of allelopathic correlations may be found that may have practical application including the field of eveloping biological measures in the struggle against the mushroom Fomes annosus. In connection with this the tests were conducted for studying the influence of phytoncides as well as of root-and leaf extracts of different plants on the growth of mycelium of Fomes annosus in culture.

MATERIALS AND METHODS

Aqueous root-and leaf extracts were prepared from the following woody-shrubbery species: Quercus borealis Michx., Robina pseudoacacia L., Carpinus betulus L., Corylus avellana L., Elaeagnus argentea Rursh., Cytisus nigricans L., Rosa rugosa Thunb., Amorpha fruticosa L., Caragana arborescens Lam., Sambucus racemosa L., Crataegus monogyna Jacq., Betula verrucosa Ehrh., Sorbus aucuparia L., Alnus incana Maench., Sorothamnus scoparius Wimm., Hippophae rhamnoides L., Malus sieboldii Rehd., and Amelanchier rotundifolia Dum.

For the purpose of preparing distillates of phytoncides the leaves of following species were taken: Helichrysum arenarium D.S., Hypericum perforatum L., Vaccinium vitis idaea L., Vaccinium myrtillus L., Calluna vulgaris Salisb., Acer negundo L. and Ranunculus acer L..

The tests for studying the effects of phytoncides and aqueous extracts of plant roots and leaves on the growth of mycelium Fomes annosus in the culture were conducted in agaric medium (brewer's must of 4 % gl. and 2% agar) poured into Petri dishes of 10 ml. For manufacturing of plant root-and leaf extracts the roots taken were freshly digged out and vital, 3-5 mm thick and also freshly-dropped leaves that were mixed by means of aknife and then destilled water of 10 times greater quantity (by weight) was poured over the leaves and kept for 24 hours. The extract obtained after its filtration was sterilized in an autoclave for 0,5 h. For manufacturing of destillates the freshly-collected plant leaves after analogous pulverization were poured with 5 times greater amount of destilled water and heated in oil-batj until 3/4 amount of added water was destilled.

Different amounts of aqueous extracts (10, 25 and 50 %) as well as distillates of plant leaves (0,1-2,0 %) were introduced into the nutritional medium immediately after the sterilization. In control dishes appropriate amounts of sterile distilled water were taken. After the

introduction of the above substances the nutritional medium was inoculated into the mycelium of radical lip. After 4-6 days the diameter of mycelium colony was measured. Every variation of the test consisted of 4-5 repetitions.

RESULTS

The investigations presented above indicated that the sensitivity of radical lip mycelium against the extracts of tree-and shrubbery roots was very different. The extracts of the roots of Robina pseudoacacia and Amorpha fruticosa indicated the greatest inhibiting effect on the growth of Fomes annosus mycelium. At the content of 10--25 % of extracts of these plants in the nutritional medium the growth of the fungus was slowed significantly and reached 50,7-71,6% that of controls. In testing dishes in which 50 % extract of Robinia pseudoacacia roots had been introduced the diameter of the fungus colony mycelium of the root lip reached only 12,9 % that of controls. The root extracts of the other trees and shrubbery showed neutral or stimulating effect on the growing processes of Fomes annosus.

Also the biologically active substances indicated nearly the same analogous effect on the growth of mycelium of Fomes annosus as the washed away water from the fallen leaves. The aqueous extracts of leaves of most tested species showed stimulating effect on the growth of Fomes annosus mycelium. Unconsiderable inhibiting effects on the growth of Fomes annosus mycelium showed the aqueous extracts of leaves of Padus racemosa, Malus sieboldii and Sorothamnus scoparius. However, clearly expressed inhibiting effect (decrease of the diameter of Fomes annosus mycelium colony up to 51-79 % if compared with that of controls) was observed only in those variations of tests where 50 % of aqueous extracts of the above plants were introduced.

Of theleaf destillates of plants tested clearly expressed fungicidal effect on the growth of radial lip of mycelium showed only the phytoncides Ranunculus acer. The everage diameter of the Fomes annosus mycelium colony in the nutritional media where 1% distillates of tall-buttercup was introduced reached only 5 % if compared with that of controls, and in the media, where the concentration of distillates Ranunculus acer was 2 %, the growth of radical lip of mycelium was not observed. The distillates of leaves of all above tested plants showed the stimulizing effect on the growth of Fomes annosus mycelium in most of the cases, but not an inhibiting one.

DISCUSSION

The research results obtained make it possible toattribute Robinia pseudoacacia and Amorpha fructicosa to the most stable plants against Fomes annosus and recommend it as the best mixture components in the cultures of coniferous species, formed on soils left after long time agricultural use. From the general of other woody-and shrubbery species tested positive effect on the stability of coniferous stands against Fomes annosus may show Padus racemosa, Malus sieboldii and Sorothamnus scoparius. The forest bedding formed of leaves of the above plants may be capable to oppress the vital processes of Fomes annosus.

The leaves of Ranunculus acer as containing phytoncides possessing strongly expressed inhibiting effect on the growth of Fomes annosus mycelium may be used as very perspective initial substance for the production of vegetative antibiotics suitable for fighting against the above fungus, and maybe, with some other kinds of plant diseases.

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СКОРОСТЬ РОСТА И АКТИВНОСТЬ ОКИСЛИТЕЛЬНЫХ ФЕРМЕНТОВ РАЗНЫХ ШТАММОВ КОРНЕВОЙ ГУБКИ [FOMITOPSIS ANNOSA (FR.) KARST.]

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ВВЕДЕНИЕ

Известно, что корневая губка способна паразитировать на многих древесных породах, вызывая у них загнивание корневой системы. Характер поражения деревьев и продолжительность заболевания у разных древесных пород неодинаковы. У таких пород как сосна, лиственница и другие гниль от корневой губки вверх по стволу не поднимается, а окольцовывая ствол у корневой шейки, вызывает отмирание деревьев. У других пород (ель, пихта) корневая гниль заходит в ствол и распространяется вверх по стволу в виде центральной гнили до 4 - 6 метров (I, 2).

Способность корневой губки паразитировать на растущих деревьях обусловлена высокой активностью многих окислительных и гидролитических ферментов гриба. До сих пор вопрос о наличии отдельных специализированных рас корневой губки, приуроченных к развитию на отдельных древесных породах окончательно не решен. Представляло интересным провести сравнительные исследования интенсивности ростовых процес-

сов и активности окислительных ферментов штаммов корневой губки при развитии их на разных древесных породах. В данном сообщении рассмотрены результаты исследований скорости роста мицелия в чистой культуре и активности каталазы, пероксидазы и лакказы у разных штаммов корневой губки.

МАТЕРИАЛ И МЕТОДЫ

Нами были собраны плодовые тела корневой губки на одном участке в смешанном хвойно-лиственном насаждении 40 - летнего возраста в Станьковской лесной даче Минского лесхоза (БССР), на котором наблюдалось одновременное поражение деревьев сосны, ели и березы корневой гнилью. Для исследования также были взяты плодовые тела корневой губки из чистых по составу насаждений сосны Негорельского учебно-опытного лесхоза и ели Прилукской лесной дачи Минского лесхоза. Культуры штаммов корневой губки были получены из мякоти плодовых тел гриба, собранных с пораженных деревьев в летний период.

Для изучения интенсивности ростовых процессов и активности окислительных ферментов мицелий гриба выращивали на 6%-ном пивном сусле в колбах Эрленмейера на биологической качалке при 25°С в течение I8 (сосновые штаммы) и 30 дней (еловые и березовые штаммы). Скорость линейного роста мицелия гриба определяли на агаризованном пивном сусле, а также наблюдали обрастание древесных блоков мицелием разных штаммов корневой губки.

Активность каталазы измеряли по способу, основанному на титрометрическом определении количества разложившейся перекиси водорода за определенный промежуток времени под действием ферментного раствора (3). Об активности пероксидазы судили по изменению интенсивности окраски продукта окисления о-дианизидина перекисью водорода, образованного при действии пероксидазы (4). Единица активности каталазы и пероксидазы соответствовала количеству фермента, катализирующего превращение I микромоль перекиси водорода за I минуту. Активность ферментов рассчитывалась на I00 мг мицелия (по сырому весу) или на I00 мл культуральной жидкости. Для измерения активности лакказы был использован колориметрический метод (5). Инкубационная смесь содержала 5 мл 0,5% раствора пирогаллола, 2 мл культуральной жидкости, I мл фосфатного буфера pH 7,6. Лакказная активность выражалась величиной поглощения при 500 ммк.

РЕЗУЛЬТАТЫ

Как показали наши исследования, имеются существенные различия в характере и скорости роста мицелия, фермента-

тивной системе разных штаммов корневой губки, растущих на искусственной питательной среде. Штаммы корневой губки, выделенные с сосновых деревьев, характеризовались пушистым белоснежным мицелием. Воздушный мицелий штаммов, выделенных с деревьев ели и березы, был желтовато-кремовым бархатистым.

Сосновые штаммы обладали более быстрым (в I,5-2 раза) линейным ростом, чем еловые и березовые штаммы. Уже на 9 сутки выращивания вся поверхность питательной среды была покрыта мицелием корневой губки, выделенной из сосновых деревьев. Мицелий березовых штаммов достигал краев чашек Петри на II-I2 сутки, а еловых штаммов - на I4 сутки. Сосновые штаммы более интенсивно накапливали биомассу мицелия при выращивании на жидкой питательной среде, чем еловые и березовые (табл. I и 2).

Таблица I.— Накопление биомассы мицелия у еловых и березовых штаммов корневой губки

Штаммы гриба	Вес мицелия в мг						
	Время выращивания гриба в сутках						
	5	I0	I5	20	25	30	35
Еловый (Станьково) № 5	35	I08	I98	267	273	267	254
Еловый (Прилуки) № I2	I9	90	I59	289	343	320	272
Березовый (Станьково) № 8	33	I30	244	300	36I	3I7	309
Березовый (Станьково) № I0	I8	II7	23I	307	279	267	2I9

У сосновых штаммов максимальное накопление биомассы мицелия происходило на I5 сутки и достигало 623-748 мг (по сухому весу), а у еловых и березовых - на 25 сутки вес мицелия был в пределах 273-36I мг при начальной pH питательной среды равной 5,9. Во время роста гриба происходило подкисление среды. Конечное значение pH культуральной жидкости было 4,4. Обрастание древесных кубиков происходило быстрее мицелием еловых и березовых штаммов.

Еловые и березовые штаммы обладали большим набором окислительных ферментов и более высокой их активностью по сравнению с сосновыми (табл. 2 и 3). Активность ферментов изменялась с возрастом культуры гриба. Еловые и березовые

Таблица 2.— Накопление биомассы мицелия и активность окислительных ферментов сосновых штаммов корневой губки

Штаммы корневой губки	Вес мицелия в мг						Каталаза (I микромоль H_2O_2 за I мин.)				Лакказа (Поглощение при 500 ммк)			
	Время выращивания гриба в сутках													
	3	6	9	I2	I5	I8	3	6	9	I2	3	6	9	I2
Сосновый (Станьково) № I	2I5	392	448	654	748	589	I32	93	30	II	60	75	20	0
Сосновый (Станьково) № 2	264	480	52I	584	692	643	54	5I	40	8	I00	I20	I0	0
Сосновый (Негорелое) № 2I	303	347	395	463	623	604	422	207	I96	I40	90	II0	20	0

Таблица 3.— Активность окислительных ферментов еловых и березовых штаммов корневой губки

Штаммы корневой губки	Каталаза (I микромоль H_2O_2 за I мин.)				Пероксидаза (I микромоль H_2O_2 за I мин.)								Лакказа (Поглощение при 500 ммк)			
					Внутриклеточная				Внеклеточная							
	Время выращивания гриба в сутках															
	5	I5	25	35	5	I5	25	35	5	I5	25	35	5	I5	25	35
Еловый (Станьково) № 5	487	2524	I092	7	0,I	0,5	I,5	0,0I	0	I0,8	44	43	45	I59	303	204
Еловый (Прилуки) № I2	3I4	2667	I4II	I598	0,I	0,5	I,6	0,09	0	5,7	55	35	0	I02	405	3I5
Березовый (Станьково) № 8	607	I75I	I45I	I045	0,02	0,3	I,5	0,03	0	I6,5	46	I3	0	I59	366	240
Березовый (Станьково) №I0	504	2664	II78	I5	0,08	0,4	0,6	0	0	I5,6	34	29	I2	I98	279	264

штаммы характеризовались активной пероксидазой как в мицелии, так и в культуральной жидкости. У сосновых штаммов активность пероксидазы отсутствовала. В мицелии еловых и березовых штаммов активность пероксидазы проявляется уже на 5 сутки, достигая максимальной величины на 20 сутки во время наибольшего накопления биомассы мицелия, к 35 суткам активность пероксидазы отсутствовала. Особенно высокий уровень пероксидазной активности наблюдался в культуральной жидкости. Биосинтез и выделение фермента в культуральную среду интенсивно происходило в течение двух недель, начиная с 15 суток роста мицелия. При дальнейшем культивировании гриба отмечалось снижение пероксидазной активности, которая почти полностью исчезала на 40 сутки роста мицелия.

Мицелий всех исследуемых штаммов корневой губки обладал внутриклеточной каталазой. Штаммы, выделенные с деревьев ели и березы, характеризовались каталазной активностью в 6-7 раз превышающей активность сосновых штаммов. Биосинтез каталазы у еловых и березовых штаммов достигал максимального значения на 15 сутки роста мицелия. У сосновых штаммов максимум в биосинтезе этого фермента отмечен в первые дни роста мицелия (3 сутки). Следует отметить на существенные различия в активности каталазы между отдельными сосновыми штаммами. Наибольшую активность каталазы имел штамм № 21, взятый из чистых сосновых культур Негорельского учебно-опытного лесхоза.

Активность внеклеточной лакказы наблюдалась у всех штаммов корневой губки. Еловые и березовые штаммы характеризовались более высокой активностью лакказы по сравнению с сосновыми. У них образование и выделение фермента в культуральную среду происходил наиболее интенсивно на 25-30 сутки роста мицелия. У сосновых же штаммов процессы биосинтеза лакказы активно проходили в молодом мицелии (3-6 суточная культура). Штаммы корневой губки, обладающие более низкой каталазной и пероксидазной активностью, как правило, имели более высокую активность внеклеточной лакказы.

ОБСУЖДЕНИЕ

Проведенные исследования показывают, что штаммы корневой губки, выделенные из деревьев сосны, ели и березы, произрастающих в чистых и смешанных по составу насаждениях, различаются по некоторым культуральным признакам, скорости роста, по набору окислительных ферментов и степени их активности при выращивании штаммов на искусственной питательной среде. Сосновые штаммы обладали более быстрым ростом и накоплением биомассы мицелия, чем еловые и березовые. При развитии корневой губки на деревьях ели и березы наблюдался усиленный биосинтез внеклеточной пе-

роксидазы, в то время как у сосновых штаммов пероксидазная активность не была отмечена как в мицелии так и в культуральной жидкости. Еловые и березовые штаммы характеризовались также повышенной активностью лакказы. Штаммы корневой губки из чистых по составу сосновых и еловых насаждений имели более высокие показатели активности отдельных окислительных ферментов, чем аналогичные штаммы из смешанных насаждений.

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RATE OF GROWTH AND ACTIVITY OF OXIDIZING ENZYMES OF DIFFERENT FOMITOPSIS ANNOSA STRAINS

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There have been studied the linear growth, accumulation of biomass and activity of peroxidase, laccase and catalase in Fomitopsis annosa strains isolated from pine, fir and birch trees in pure and mixed plantations of the Byelorussia. It has been proved that pine strains are characteristic of a more rapid linear growth (1,5 - 2 times) and larger mycelium biomass accumulation than fir and birch strains. The latter are characterised by active peroxidase in a mycelium and culture solution. In the pine strains peroxidase was missing. The activity of catalase and laccase was higher in the fir and birch strains. It has also been noted that the activity in Fomitopsis annosa strains of pure plantations is higher than in mixed ones.

ПОТРЕБНОСТЬ *Fomitopsis annosa* В РАЗЛИЧНЫХ ОРГАНИЧЕСКИХ И МИНЕРАЛЬНЫХ ИСТОЧНИКАХ ПИТАНИЯ

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ВВЕДЕНИЕ

Массовое поражение *Fomitopsis annosa* хвойных пород в лесах Северного Полушария представляет собой одну из первоочередных проблем в лесной фитопатологии. Успешное её решение во многом связано с познанием биологии и физиологии гриба, в том числе вопросов питания *F.annosa*. Анализ литературных сведений свидетельствует о том, что потребность этого гриба в различных органических соединениях и минеральных элементах до сих пор изучены весьма слабо, хотя Nord и Sciarini /5/, Фёдоров и Стайченко /4/ поставили опыты по выяснению пригодности некоторых групп органических соединений для *F.annosa*.

Учитывая особое положение углерода среди элементов, необходимых для жизнедеятельности гриба, нами были поставлены опыты по определению степени пригодности для питания гриба различных групп углеводов и солей органических кислот. Отсутствие данных о возможности использования *F.annosa* различных азотистых оснований или нуклеотидов и о потребности *F. annosa* в элементах минерального питания побудило поставить нас соответствующие эксперименты.

МАТЕРИАЛ И МЕТОДИКА

В качестве источников углеродного питания для F. annosa испытано около 30 углеводов, относящихся к различным группам - моно-, ди-, три- и полисахаридам, а также ряд органических кислот, образующихся в цикле Кребса, играющих многообразную роль в метаболизме грибов и заслуживающих особого интереса с точки зрения их физиологии. Исследования велись в 100-мл колбах на жидкой и агаризированной средах /аспарагин - 1 г, глюкоза или другой углевод - 10 г, KH_2PO_4 - 2 г, $MgSO_4 \cdot 7H_2O$ - 0,5 г, вода дистиллированная - - 1 л/. При изучении возможности усваивания F. annosa солей органических кислот в качестве источника углерода бралось 3 г соответствующей соли. Для определения степени усвоения азотистых оснований или нуклеотидов количество азотсодержащих веществ составляло 1 г на 1 г среды. Во всех случаях в качестве контроля служила среда без соответствующего источника углерода или азота. В опыте с изучением потребности F. annosa в минеральных элементах использовалась среда следующего состава: глюкоза - 15 г, NH_4NO_3 - 15 г, KH_2PO_4 - - 1 г /KCl - 1 г/, $MgSO_4 \cdot 7H_2O$ - 1 г, $CaCl_2$ - 0,1 г, NaCl - - 0,1 г, $FeCl_2$ - 0,6 г $CuSO_4$ - 0,23 мг, $Na_2B_4O_7$ - 0,045 мг, Na_2MoO_4 - 0,043 мг, $MnCl_2$ - 0,46 мг, $ZnSO_4 \cdot 7H_2O$ - 4,3 мг, картофельная мезга - 3 г, вода дистиллированная - до 1 л, агар - 30 г.

Скорость роста гриба и количество образовавшегося мицелия учитывалось через 5 и 10 суток после засева среды. По истечении указанных сроков мицелий, выращивавшийся на жидкой среде, отфильтровывали, высушивали до постоянного веса и взвешивали.

РЕЗУЛЬТАТЫ

Использование углеводов F. annosa

Инокуляция питательной среды F. annosa свидетельствует о том, что гриб способен усваивать различные группы углеводов, хотя степень их усвоения далеко неодинакова /таблица 1/.

Таблица 1. Рост F. annosa на питательных средах с различными углеводами

Наименование углеводов	Рост мицелия							
	сухой вес на жидкой среде через				Диаметр колонии на агаризированной среде через			
	5 суток		10 суток		5 суток		10 суток	
	в мг	в % к контролю	в мг	в % к контролю	в мм	в % к контролю	в мм	в % к контролю
1	2	3	4	5	6	7	8	9
Контроль	4,4	100,0	25,7	100,0	16	100,0	38	100,0

Наименование углеводов	Рост мицелия: сухой вес на жидкой среде, 5 суток, в мг	в % к контролю	10 суток, в мг	в % к контролю	Диаметр колонии на агаризированной среде через 5 суток, в мм	в % к контролю	10 суток, в мм	в % к контролю
I	2	3	4	5	6	7	8	9
Моносахариды								
Д-Эритроза	15,1	343,1	162,5	632,2	26	162,5	70	184,2
Д-Аллоза	13,0	295,4	112,5	437,7	23	143,7	59	155,2
Аллометилоза	12,7	288,6	130,4	507,3	22	137,5	51	134,2
Д-Апиоза	15,0	340,9	142,3	553,7	20	125,0	48	126,3
Д-Арабиноза	16,3	370,4	157,2	616,3	28	175,0	80	210,5
Д-Галактоза	15,8	359,0	153,0	595,3	28	175,0	72	189,4
Д-Глюкоза	23,7	538,6	208,3	810,5	34	212,5	90	236,8
Д-Ксилулеза	14,7	334,0	122,7	477,4	29	181,2	86	226,3
Д-Ксилоза	15,1	343,1	142,2	553,3	32	200,0	85	223,7
Д-Ликсоза	12,0	272,7	100,8	392,2	25	156,2	75	197,3
Д-Маннноза	14,5	329,5	110,7	430,7	32	200,0	85	223,7
Д-Маннокетогентулёза	12,8	290,9	118,0	459,1	24	150,0	70	184,2
Д-ононит	8,2	186,3	73,5	286,0	21	131,2	46	121,0
-рамноза	14,2	322,7	137,0	533,0	30	187,5	80	210,5
Д-рибоза	17,7	402,2	160,2	623,3	35	218,7	90	236,8
Д-рибулёза	17,0	386,3	168,2	654,4	37	231,2	90	236,8
Д-седогентулёза	18,1	411,3	172,8	672,3	34	212,5	90	236,8
-сорбоза	10,1	229,5	82,3	320,2	18	112,5	52	136,8
Д-тагатоза	8,7	197,7	72,1	280,5	22	137,5	50	131,5
Д-талоза	7,6	172,7	62,0	241,2	24	150,0	56	147,3
Д-треоза	7,0	159,0	81,2	315,9	20	125,0	48	126,3
Д-фруктоза	16,8	381,8	150,2	584,4	32	200,0	90	236,8
Дисахариды								
Гентибиоза	12,7	288,6	109,8	427,2	22	137,5	50	131,5
Мальтоза	11,1	252,2	98,2	382,1	23	143,7	58	152,6
Маннобиоза	13,7	311,3	112,6	438,1	23	143,7	62	163,1
Мелибиоза	12,8	290,9	114,7	445,9	21	131,2	58	152,6
Неотрегалоза	14,0	318,1	111,0	431,9	21	131,2	64	168,4
Сахароза	15,2	343,1	126,7	493,0	20	125,0	65	171,0
Тураноза	10,0	227,2	96,3	374,7	18	112,5	48	126,3
Целлобиоза	13,0	295,4	120,7	469,6	23	143,7	57	150,0
Эпигентибиоза	9,7	220,4	80,2	312,0	20	125,0	51	134,2
Эпилактоза	7,6	172,7	66,5	258,7	22	137,5	63	165,7
Лактоза	11,8	268,1	108,5	422,1	19	118,7	65	171,0

I	2	3	4	5	6	7	8	9
				Трисахариды				
Раффиноза	I6,7	379,5	I74,0	677,0	25	I56,2	76	200,0
Галактотриоза	I5,2	343,I	I57,0	6I0,8	27	I68,5	72	I89,4
Маннотриоза	I3,2	300,0	II7,6	457,5	23	I43,7	67	I76,3
Паноза	9,0	204,5	80,I	3II,6	24	I50,0	70	I84,2
Трегалоза	I8,7	425,0	I97,2	767,3	32	200,0	80	2I0,5
Фукозидолактоза	8,0	I8I,8	79,I	307,7	22	I37,5	58	I52,6
Целлотриоза	9,8	222,7	82,3	320,2	20	I25,0	55	I44,7
				Полисахариды П порядка				
Амилоза	I8,9	429,5	I90,2	740,0	29	I8I,2	75	I97,3
Крахмал	I5,6	354,5	I49,0	579,7	26	I62,5	70	I84,2
Гликоген	I8,7	425,0	I73,5	675,0	27	I68,5	72	I89,4
Лихенин	I0,I	I29,5	79,5	309,3	I7	I06,2	48	I26,3
Инулин	I7,8	404,5	I62,6	632,6	24	I50,0	65	I7I,0

Наиболее интенсивные ростовые процессы наблюдались на питательных средах, содержащих моносахариды - Д-глюкозу, Д-рибозу, Д-рибулезу, Д-седогентулезу, Д-фруктозу, Д-ксилозу, Д-ксилулезу и др. Вес сухого мицелия в этом случае увеличивался в 3-6 раз в сравнении с контрольным вариантом. Другие моносахариды также интенсивно использовались, хотя некоторые, как Д-галоза, Д-треоза, Д-инонит, Д-аллоза, алло-метилоза, L-сорбоза - оказывали более слабое воздействие на ростовые процессы гриба. Кстати, L -сорбозу многие грибы или совсем не используют, или усваивают её очень слабо. Дисахариды также вполне удовлетворительно усваиваются, хотя в целом рост гриба на этих средах был несколько заниженным. Так, на средах, содержащих туранозу, гентабиозу, эпигентибиозу скорость ростовых процессов на агаровой среде была лишь на 0,6-0,8 раза выше, чем в контроле. Из трисахаридов наиболее существенное воздействие воздействие на рост F. annosa оказали -трегалактоза, раффиноза, галактотриоза, остальные - по интенсивности усвоения грибом примерно соответствовали дисахаридам. Полисахариды отличаются весьма хорошим усвоением, хотя некоторые из них /лихенин/ мало доступны для F. annosa.

Из полученных результатов также следует, что накопление массы мицелия на жидкой среде в сравнении с контролем в большинстве случаев протекает более интенсивно, чем рост гриба на твердой среде, эта особенность была также отмечена Фёдоровым и Стайченко /4/. Результаты наших работ, в основном, подтвердили выводы, сделанные этими авторами об использовании корневой губкой различных углеводов для питания, хотя следовало бы сделать некоторые уточнения. Так, судя по таблице, приводимой названными авторами, характеризующей рост мицелия на твердой среде, внесение в питательную среду

моносахарида L -сорбозы привело к значительному, более чем в 3 раза, замедлению роста мицелия, в сравнении с контролем. Определенное ингибирующее влияние на рост мицелия оказывали сахара - мальтоза, Д-фруктоза. Вместе с тем, опыты этих авторов по выращиванию F. annosa на жидкой питательной среде свидетельствуют о довольно интенсивном усвоении грибом указанных сахаров. Видимо результаты исследований по выращиванию гриба на твердых и жидких средах не всегда являются тождественными, хотя в принципе они в достаточной степени соответствуют друг другу.

Использование солей органических кислот.

Органические кислоты в зависимости от pH клеточного сока могут находиться как в свободном состоянии, так и в виде солей, причем существует определенная избирательность F.annosa в усвоении органических кислот, которые преобладают при более низких значениях pH /таблица 2/.

Таблица 2. Рост F. annosa на питательных средах с органическими кислотами

Наименование органических кислот /солей/	Рост мицелия							
	Сухой вес на жидкой среде через				Диаметр колонии на агаризированной среде через			
	5 суток		I0 суток		5 суток		I0 суток	
	в мг	в % к контролю	в мг	в % к контролю	в мм	в % к контролю	в мм	в % к контролю
I	2	3	4	5	6	7	8	9
Контроль	4,8	I00,0	36,8	I00,0	I7	I00,0	40	I00,0
Глиоксилевокислый натрий	9,7	202,0	92,0	250,0	36	2II,7	80	200,0
Глицериновокислый кальций	8,9	I85,4	87,9	238,8	3I	I82,9	76	I90,0
Пировиноградно-кислый калий	II,6	24I,6	II7,0	3I7,9	36	2II,7	80	200,0
Пропионовокислый кальций	6,0	I25,0	68,5	I86,I	24	I4I,I	52	I30,0
Маслянокислый натрий	5,0	I04,I	42,8	II6,3	I8	I05,8	42	I05,0
Молочнокислый кальций	I0,5	2I8,7	I27,3	345,9	34	200,0	80	200,0
Муравьинокислый калий	4,2	87,5	30,6	83,I	I4	82,3	30	75,0
Уксуснокислый натрий	3,0	62,5	26,7	72,5	I0	58,8	28	70,0
Янтарнокислый калий	8,8	I83,3	73,6	200,0	27	I58,8	70	I75,0

I	2	3	4	5	6	7	8	9
Фенилпировиноградноки-слый натрий	9,0	I87,5	87,5	237,7	30	I76,4	75	I87,5
Лимоннокислый натрий	8,2	I70,8	52,3	I44,8	29	I70,5	68	I70,0

Лучше всего усваиваются соединения, входящие в цикл Кребса и цикл глиоксилевой кислоты, которые гриб получает при ферментативном разложении углеводов. Наибольшее накопление мицелия имело место в средах, содержащих молочнокислый кальций, пировиноградноки слый калий, глиоксилевокислый натрий, янтарнокислый калий. Увеличение массы мицелия в этом случае наблюдалось в 2,5-3 раза,в сравнении с контролем. Угнетающее влияние на накопление массы гриба оказали муравьинокислый калий и уксуснокислый натрий. Примерно аналогичная зависимость обнаруживалась и в скорости роста мицелия как на твердой, так и на жидкой среде.

Результаты опытов свидетельствуют о том, что органические кислоты, образующиеся в ходе аэробного дыхания, легко усваиваются грибом и служат важным резервом в углеродном питании. Вместе с тем, соли некоторых органических кислот, образование которых не связано с циклом Кребса и глиоксалатным циклом, оказывают определенное ингибирующее воздействие на ростовые процессы. Недоступность этих кислот на наш взгляд можно объяснить тем, что образование их происходит главным образом в ходе брожений, вызываемых различными микроорганизмами, с которыми F. annosa может находиться в антагонистических отношениях. Именно наличие этих отношений не позволило F. annosa приспособиться в ходе эволюционного развития к усвоению углерода указанных органических кислот.

Усвоение F. annosa азотистых оснований и нуклеотидов

Рост мицелия F. annosa на средах, содержащих азотистые оснований и нуклеотиды намного превосходит рост гриба в контроле /таблица 3/.

Таблица 3. Рост F. annosa на среде с азотистыми основаниями и нуклеотидами

Источники азотного питания	Рост мицелия							
	Сухой вес на жидкой среде через				Диаметр колонии на агаризированной среде через			
	5 суток		10 суток		5 суток		10 суток	
	в мг	в % к контролю	в мг	в % к контролю	в мм	в %к контролю	в мм	в % к контролю
Аденин	8,0	210,5	67,9	543,2	23	287,5	60	272,7
Гуанин	7,6	200,0	50,3	400,2	22	275,0	56	254,5
Цитозин	8,8	231,5	70,2	561,6	26	325,0	65	295,4
Урацил	9,2	242,1	77,5	620,0	30	375,0	68	309,0
Тимин	8,8	231,5	71,0	568,0	25	312,5	59	268,1
Аденозин-5-дифосфат натрия	6,5	171,0	45,7	365,6	20	250,0	48	218,1
Гуанозин-5-монофосфат натрия	7,7	202,6	49,5	396,0	20	250,0	52	236,3
Цитидин-5 дифосфат бария	8,0	210,5	61,9	495,2	23	287,5	58	263,6
Уридин-5-монофосфат натрия	8,4	221,0	68,0	544,0	24	300,0	60	272,7
Тимидин-5-дифосфат бария	8,1	213,1	56,6	452,8	24	300,0	66	300,0
Аспарагин	6,8	152,6	34,1	272,8	20	250,0	56	254,5
Контроль /без источника азотного питания/	3,8	100,0	12,5	100,0	8	100,0	22	100,0

Более значительно отличия в скорости роста проявляются на жидкой среде, где скорость роста мицелия была в 2,5-3 раза выше, чем в контроле. Показательно, что гриб по существу одинаково интенсивно усваивает как пуриновые, так и пиримидиновые основания, входящие в состав нуклеиновых кислот. Однако, некоторая избирательность в поглощении оснований все же прослеживается. Так, рост мицелия на среде, содержащей урацил, был быстрее, чем на среде с гуанином примерно на 50%. Рост гриба на средах с пуриновыми и пиримидиновыми основаниями был несколько выше, чем на средах, содержащих нуклеотиды. Очевидно это связано с тем, что нуклеотиды, являясь более сложными соединениями, подвергаются ферментативному расщеплению на пуриновые и пиримидиновые основания, сахара - рибозу или дезоксирибозу и фосфорную кислоты. И только после этого происходит их усвоение. Учитывая то обстоятельство, что в среде содержались источники сахара и фосфора и организм гриба недостатка в них не испытывал, стимулирующего эффекта на ростовые процессы оказать они не могли.

Если сопоставить скорость роста F. annosa на средах с пуриновыми и пиримидиновыми основаниями и нуклеотидами со скоростью роста на среде, содержащей аспарагин /взятый для сравнения/, можно констатировать определенное в I,5-2 раза ускорение ростовых процессов в первом случае. Однако следует иметь в виду, что аспарагин не относится к числу особо благоприятных для гриба источников питания азотом. Поэтому ускорение роста на средах, содержащих названные выше соединения, нельзя назвать сколь-нибудь заметным. Более того, сопоставляя полученные результаты с данными Фёдорова и Стайченко /4/ об использовании грибом аминокислот в качестве источников азотного питания, напрашивается вывод о том, что аминокислоты /за редким исключением/ сильнее ускоряют ростовые процессы и, следовательно, являются более доступными для F.annosa.

Как сообщает Кретович /2/ пуриновые и пиримидиновые основания играют важную роль в качестве стимуляторов роста растений и микроорганизмов. Однако приведенные исследования не дают основания к такому заключению относительно влияния их на организм F. annosa . Тем не менее при внесении небольших доз пуриновых и пиримидиновых оснований в полную питательную среду можно ожидать более выраженный стимулирующий эффект.

Потребность F. annosa в элементах минерального питания.

Исключение из состава среды отдельных минеральных элементов не вызвало полного отсутствия роста F. annosa ни в одном из вариантов, хотя ингибирование роста в некоторых вариантах было весьма заметным /таблица 4/.

Таблица 4. Рост F. annosa на синтетической среде с исключением минеральных элементов

Исключаемые элементы /компоненты/	Рост мицелия							
	Сухой вес на жидкой среде через				Диаметр колонии на агаризированной среде через			
	5 суток		I0 суток		5 суток		I0 суток	
	в мг	в % к контролю	в мг	в % к контролю	в мм	в % к контролю	в мм	в % к контролю
I	2	3	4	5	6	7	8	9
NH_4NO_3	2,2	68,7	I4,5	63,5	I5	60,0	52	74,3
KH_2PO_4	2,0	62,5	I2,0	52,6	I2	48,0	40	57,I
KCl /вместо KH_2PO_4/	2,I	65,6	I2,6	55,2	I2	48,0	45	64,3
$MgSO_4$	I,2	59,3	I0,3	45,I	I0	40,0	3I	44,3
$CaCl_2$	2,2	68,7	I2,9	56,5	I4	56,0	54	77,I

I	2	3	4	5	6	7	8	9
$FeCl_2$	2,0	62,5	I2,3	53,9	I3	52,0	48	68,5
$CuSO_4$	2,8	87,5	I5,3	67,I	I7	68,0	52	74,3
$Na_2B_4O_7$	3,0	93,7	20,8	9I,2	I8	72,0	63	90,0
Na_2MoO_4	2,8	87,5	2I,2	9I,9	I8	72,0	62	88,5
$MnCl_2$	3,I	96,8	22,0	96,4	23	92,0	70	I00,0
$ZnSO_4$	2,0	62,5	II,8	5I,7	II	44,0	44	62,8
Картофельный отвар	I,8	56,2	I0,I	44,3	9	36,0	30	42,8
Контроль /полная среда/	3,2	I00,0	22,8	I00,0	25	I00,0	70	I00,0

Весьма слабый рост F. annosa отмечен на среде с отсутствием калия и фосфора; на жидкой среде сухой вес мицелия составлял лишь 52,6 % от контроля, а на агаризированной 57,I %. В варианте, где отсутствовал только фосфор, рост гриба был также слабым. Наблюдавшееся торможение ростовых процессов объясняется важным физиологическим значением фосфора, который входит в состав нуклеопротеидов и участвует в процессах фосфорилирования, играющих первостепенную роль в обмене веществ. Отсутствие в среде калия, находящегося в свободном состоянии, оказывает менее значительное влияние на ростовые процессы гриба. Очевидно это связано с тем, что у грибов калий частично может быть заменен натрием /Беккер /I/, который находился в среде. Существенно влияет на рост гриба исключение из среды магния. Хотя магний в мицелии не образует стойких органических соединений, тем не менее он участвует в активизации ряда ферментов дыхания. Кальций оказывает более слабое влияние на ростовые процессы, хотя в золе некоторых грибов на долю кальция приходится до 50 %. Сухой вес мицелия на среде без кальция был на 25–35 % ниже, чем в контроле. Последнее связано с тем, что кальций играет роль нейтрализатора органических кислот и оказывает воздействие на снижение проницаемости протоплазмы.

Существенно, на 35–40 %, снижена скорость ростовых процессов мицелия и на среде без цинка. Последний входит в состав ферментов, участвующих в превращениях продуктов расщепления углеводов в составные части клетки, а также предотвращает образование избыточного количества органических кислот. Столь же существенно снижает ростовые процессы отсутствие железа. Этот микроэлемент требуется грибам в наибольшем количестве, так как входит в состав каталазы, пероксидазы и цитохромоксидазы и активирует ряд других ферментов.

Определенное ингибирование роста имело место и на среде с отсутствием меди. Хотя потребность в этом элементе ничтожна, тем не менее, поскольку медь входит в состав полифенолоксидазы, лакказы, оксидазы, аскорбиновой кислоты и других очень важных для обмена ферментов, исключение меди тормозит ростовые процессы гриба.

Исключение из среды бора и молибдена слабо сказалось на росте мицелия, а рост гриба на среде без марганца был

практически одинаковым с контролем. Между тем известно, что указанные микроэлементы входят в состав ферментов и выполняют ряд других физиологических функций. Очевидно по отношению к этим элементам существует известная взаимозаменяемость. Не исключено также, что какое-то количество этих элементов присутствовало в качестве примесей в используемых солях. Характерно также, что рост гриба без картофельного отвара сильно угнетался.

Из сказанного вытекает, что F. annosa наибольшую отзывчивость проявляет на внесение в питательную среду фосфора, калия, магния, кальция, железа, цинка и меди. Очевидно эти элементы относятся к числу наиболее важных в питании гриба.

SUMMARY

F.annosa can assimilate different groups of carbohydrates. The most intensive growth processes have been observed in nutrient media containing monosaccharids, though D-inosit, D-annosa, L-sorbosa exerted rather weak influence upon the growth processes. Di-saccharids and three-sacharids are quite satisfactorily adopted by the fungus, but the growth of the fungus on these media was somewhat understated, especially with the content of thuranose, hectabiose, -tregalactose, raffinose. It was found that lichenin was the most accessible for the fungus. The organic acids salts formed in the process of aerobic breathing are easily assimilated by the fungus though the fungi which have no connection with the cycle of Krebs and that of glioxalat make some inhibiting influence upon the process of growth. F.annosa can use the nitrous base and nucleotids in the quality of the sources of nutrition. Out of the number of mineral elements F.annosa is the most susceptive to phosphorus, potassium, magnesium, calcium, iron, zinc and copper.

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ABOUT THE OCCURRENCE OF ECOTYPES OF THE SPECIES FOMES ANNOSUS (Fr.) Cooke

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INTRODUCTION

An occurrence of races or ecotypes of the fungus Fomes annosus (Fr.) Cooke has not yet been observed, even though his worldwide occurrence does not exclude this possibility (Etheridge 1955, Braun 1958, Cowling a. Kelman 1964). The development of races can be triggered by parasexuality, anastomoses of dicaryiontic hyphes - if heterocaryonts are present - as well as by selection ascribed to environmental influences (Hansen a. Smith 1932, Esser a. Kuenen 1965). To prove a race development we can choose several methods, two will be outlined here:

1. we can try experimentally to alter specific characteristics and the behaviour of fungi under extreme environmental conditions, or
2. as many characteristics as possible will be investigated concerning their quality, quantity and constance.

Since the objective of all these investigations is to obtain methods, chemical or biological, for precautionary measures against a Fomes annosus attack, the physiological characteristics, connected with the growth and the damaging intensity of the fungus, receive priority in this study. Therefore special attention has to be given to physiological differences and to identical features likewise.

MATERIALS AND METHODS

In our investigation we chose the second method and tested several physiological characteristics of 15 assorted Fomes annosus strains. They were isolated from spruce wood grown on either calcareous or lime deficient soils. Long-term-strains have been cultivated on nutrient substrate (malt agar) since 1955/56 and short-term-strains since 1966/68 (70). We used conidiospores (macrospores). They were easier to get than basidiospores and seem to allow careful conclusions on the behaviour of basidiospores (Zycha a. Kliefoth 1971). The methods used were described in earlier publications (Courtois 1972 a,b).

RESULTS

1. Sporulation and conidia size

The amount of available spores is a measurement for the easiness with which the fungus spreads and the infection density is a criterium for his aggressiveness. The sporulation frequency, i. e. a multiple generation sequence pro annum, makes it possible for the fungus to survive with the help of a wide variability of his characteristics. Just those spores will develop whose characteristics are to the utmost adjusted to the environmental conditions.

Sporulation of conidia is tightly correlated with the mycel growth. It appears about 3 - 10 mm behind the front zone of the mycelium. Furthermore sporulation depends on the temperature. It starts at low C-source (0,1 % malt) at about 17°C. At both dependencies differences between the strains are existing. At higher C-supply (3 % malt) short-term-strains begin sporulation already at 8°- 12°C, but long-term-strains do not. The differences are significant. At a lower temperature (+4°C) substantial differences in the beginning of spore development, measured in days, occur. They are strain specific. None of all above mentioned differences causes a race separation (Courtois 1972 a).

A reduction of the O_2-partial pressure to 60 Torr (1/18 of the normal value) lowers the spore development quantitatively or even prevents it. There isn't any hint for race specific differences either.

The conidia size is readily applied for taxonomic causes, because this morphological characteristic is in almost all cases genetically fixed. Significant differences observed between the strains do not point out a cause for race separation.

2. Conidia germination

The germination of the spores possesses probably for the adherence of the infection greater importance than the amount of spreaded spores for the aggressiveness. The germination phase is, referring to the remaining fungus stages, especially sensitive towards the environmental influences.

In all strains the germ-percentage is there higher, where the motherscultures, from which the tested conidia were harvested, grew on a naturlike substrate.Thus the influence of the nutrient substrate is made obvious, and occurs in all isolates in the same manner.

Concerning the age of the motherculture, the origin of the conidia, two groups were observed: In the first group the entire strains showed, independent of the age of the motherculture, always the same germination ability. In the second group the germ speed decreases until the age of about 18 days, to stay constant from the 20th day on.

The dependency of the germination ability from the substrate and the age of the motherculture shows strain specific characteristics but no reason to separate races.

Likewise strain specific differences occur in all isolates concerning the dependency of germination from the temperature in the germinationbed. Furthermore long-term-strains show in their optimal temperature curve a peak and short-term-strains a plateau over several temperature degrees (Fig.1). This characteristic may refer as well to the adaptability of this kind of fungus, if we postulate that long-term-strains reacted similar as short-term-strains about 17 years ago. Very interesting is the observation, that strains in a lower temperature range sometimes interrupt the decreasing of their germ-percentage suddenly. This could be the

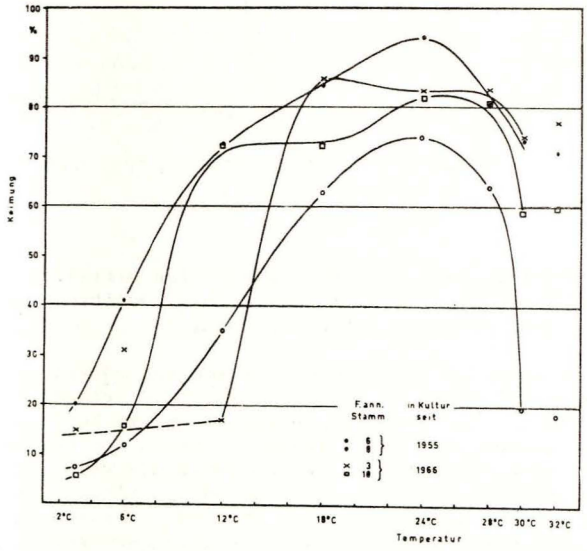

Fig. 1 Short-term-strains show germ-percentage curves with a plateau optimum between 12°-28°C and long-term-strains a peak at about 24°C.

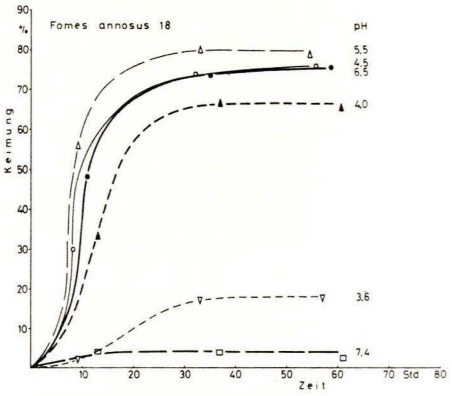

Fig. 2
Strains from calcareous soils have a higher germ-speed at about pH 5,5

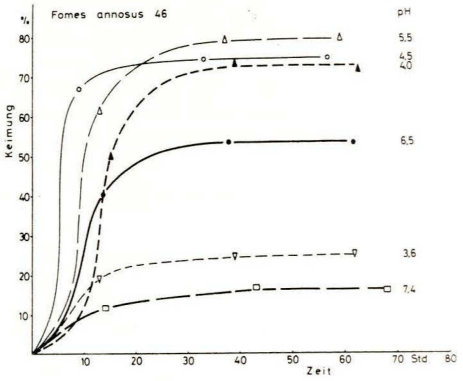

Fig. 3
Strains from lime deficient soils have a higher germ-speed at about pH 4,5

cause, heterogen pattern provided, for a wide variability. But we need more detailed knowledge of the nucleus conditions and about an up to now just presumed heterocaryosis.

In connection with the germ-percentage and the pH conditions in the germ-bed significant individual strain differences were noticed again. In a wide range of pH compatibility between pH 4-6,5 certain indications for the existence of races are not observed. On the other hand the highest germ-speed values were attained with strains from calcareous soils at about pH 5,5 (Fig.2) and from limedeficient soils at about pH 4,5 (Fig.3). Likewise was detected, that Fomes annosus strains always reach a higher germ-percentage on the germ-soil of their origin than on other soils (Courtois 1972 b). Concerning these observations criteria become obvious that indicate the formation of two ecotypes. Those were observed again in an other connection and will be discussed now.

3. Mycelgrowth

The linear growth is closely related to the occupation of living-space and the mycel weight to the decomposition of wood. Etheridge (1955) has engaged himself already 18 years ago with the first mentioned characteristic concerning race development. We took up his questioning again and can certify that linear growth does not indicate the existence of races.

In mycel growth short-term-strains produce higher weights than long-term-strains. This can be caused by the long term constant artificial culture conditions resulting in selection.

With increasing mycel weight the strains secrete increasingly H^+-ions into the solution. The secretion is pro strain generally constant. Strains from calcareous soils reach a maximal mycel weight between pH 5 - 6,9 , whereas isolates from lime deficient soils reach the maximal mycel weight at about pH 4 (Fig.4).

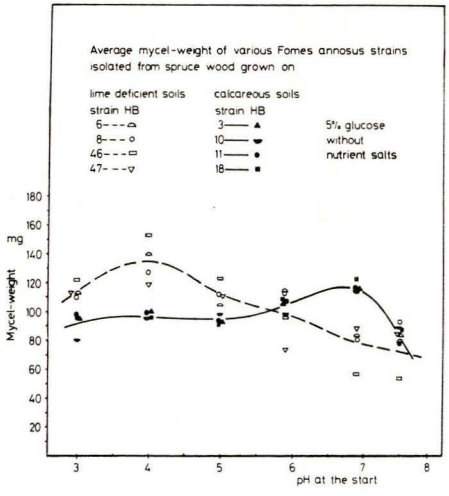

Fig. 4 Dependency of the mycel weight from the pH

Furthermore in a low acidic range (pH 5 - 6) strains from calcareous soils dispose of more H^+-ions into the metabolism secretions than isolates of lime deficient soils.

The noted results lead us to conclude that there are two ecotypes existing, one on lime deficient and the other on calcareous soils.

4. Enzyme activity and wood decomposition

Cellulase activity shows significant quantitative differences between the isolates. Long-term-strains too develop a higher cellulase activity than short-term-strains. Since our investigations about this physiological characteristic is not concluded yet, we can not make any certain statements about a possible race development at this time.

The same prevails for our investigations and results concerning wood decomposition. There we also find a wide variability between several strains. The deviations are often significant. The kind of standard deviation of the single values allows us, rather to prescribe a center of kinds than race differences.

DISCUSSION

With ecotype we define a population of individuals whose expression or alternation of some characteristics are due to environmental influences that have been handed down over some years from clone to clone. Our conception does not mean race (Stamm, Form, Biotyp) as it did for Gäumann (1951), who defined a population, evolved from a single culture, from a genetic homogen individuum. Our ecotypes still need a genetic investigation.

Setting up two ecotypes we were led by the idea, that the deviation about the mean value is more relevant than the mean value. We are in conformity with Nieman (1957) that one can look at groups of origins as to be systematically different only, if the origins to be classified , show no or just little overlapping in the deviation range.

If physiological differences are not enough to separate races Fisher (1953), we have to remark, that Nobles (1965) used ecological and physiological features for the identification of fungus kinds. It is therefore possible that physiological features may reach the same range of importance as other systematic characteristics. Above that, Schwinn (1959) holds the opinion that "die Physiologie besseren Aufschluß über Artgrenzen gegeben hat als die Morphologie".

Qualitative features are meant above. But in our lecture we deal with, besides qualitative features, also the significant differences of one feature. This, the mycel weight dependency from the solution pH of its natural site, is constant and is enlarged and completed by other criteria. I.e. culturing through the years on artificial substrate alters the dependency of sporulation from the temperature, and the dependency of the germination and the mycel weight from the pH. There the plasticity and the ability of the fungus kind to alter in vitro the used physiological features by means of selection, is easily visable. Therefore the separation of ecotypes seems tolerable.

Our knowledge of the genetic proportions of Fomes annosus is still poor. The evolution of heterocaryonts is besides other possibilities ascribed to anastomoses of dicaryontic hyphes (Hansen a. Smith 1932). If homologic nuclei in the cells are not existing, a high genetic variability is to be expected. The range of this variability within the strains and, if it overlaps or not an other one present between the strains, is for the judgement of some physiological behaviour patterns of great importance. Of those the qualitative and quantitative enzyme furnishings are more interesting because they are involved causative with the aggressiveness of the strains and their pathogenity. Should, i.e. a wide enzyme variabi-

lity arise, then there is only a small chance, to fight a Fomes annosus attack by culturing resistent tree species.

If the characteristics of the fungus Fomes annosus are not completely settled yet, so mainly because only partial courses can be conducted in an experiment. If complex connected characteristics are separated, various results may occur. Varying conclusions do not have to be the sequence. For this reason sufficient security is necessary to prevent illogical conclusions.

SUMMARY

Within the fungus kind Fomes annosus the development of two ecotypes is possible. They differ 1. in the mycel growth depending on the pH of the nutrient solution, 2. in secretion of different high amounts of H^+-ions in a moderate acidic pH range, 3. they achieve in the germ-substrate of their origin always the highest germ-percentage and 4. we find the highest germ-speed of strains from calcareous soils at about pH 5,5 and from lime deficient soils at about pH 4,5.

ZUSAMMENFASSUNG

Innerhalb der Pilzart Fomes annosus ist die Ausbildung von zwei Ökotypen möglich. Sie unterscheiden sich 1. in der Mycelentwicklung in Abhängigkeit vom pH der Nährlösung 2. darin, im schwach sauren pH-Milieu unterschiedliche Mengen H^+-Ionen auszuscheiden, 3. auf dem Keimboden ihrer Herkunft stets die höchsten Keimprozente zu erzielen und 4. liegen die Keimgeschwindigkeiten der Stämme von kalkhaltigen Böden um pH 5,5 und die von kalkarmen Böden um pH 4,5 am höchsten.

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EFFECTS OF EXTRACTIVES FROM SPRUCE ROOTS ON EXTRACELLULAR ENZYMES OF FOMES ANNOSUS (FR.) CKE.

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INTRODUCTION

Root infecting fungi, i.e. Fomes annosus, growing in the heartwood of Norway spruce stems cause a transformation of parts of the surrounding sapwood into a so-called reaction zone (12, 13). A similar transformation of the sound sapwood is found in roots very soon after fungal attack (4). Extracts from this zone, compared with those from adjacent sound sapwood, show several differences. The chemical properties of the reaction zone make it unsuitable for fungal growth.

The penetration of fungal hyphae through wood cell walls is dependent on the activity of extracellular enzymes capable of decomposing cellulose, hemicellulose, lignin and pectin. In cases where all or some of these enzymes are necessary for fungal growth, the enzyme most susceptible to inhibition becomes the limiting factor in the advancement. In this paper is shortly reported some experiments undertaken to determine the effect of extractives and pure compounds from spruce wood on extracellular enzymes produced by Fomes annosus.

MATERIALS AND METHODS

Samples of sound sapwood and reaction zone of spruce roots, attacked by Fomes annosus and also heartwood of thick unattacked roots were milled and extracted with acetone. The extract was divided into fractions soluble in light petroleum (resinous compounds), n-butanone (lignans, phenols, glucosides), and water (glucosides, carbohydrates). The extractives were dissolved in ethanol and used in concentrations corresponding to those of the fresh wood.

For enzyme preparation Fomes annosus was cultivated without shaking on Norkrans' medium (9), with 1 g asparagine, 0.5 g glucose and 30 g milled spruce heartwood/l. When the activities of cellulase, pectinase, aryl-β-glucosidase and laccase were near their maximum productions in the culture media the enzymes were precipitated with two volumes of cold acetone over night. The precipitate was made acetone free and dissolved in distilled water and diluted with appropriate buffers to the activity desired for each enzyme. Commercial horse radish peroxidase and tyrosinase (SIGMA Chemical Company) were used in some tests.

Cellulase assay: The fraction or ompound to be tested, dissolved in ethanol, was added to the enzyme-buffer solution (0.05 M Ac^- buffer, pH 5.5) in concentrations corresponding to 1, 1/3, 1/10 and 1/100 that of the fresh wood. NaN_3 was added to a concentration of 10^{-4} M to prevent oxidation of phenols by laccase, present in the enzyme preparation. Control experiments were performed with ethanol alone. The mixture was shaken vigorously and left at room temperature for at least 4 hours (8), followed by centrifugation. 5.0 ml of the supernatant was mixed with 5.0 ml of CMC solution in the same buffer and incubated at 25°C for 15 minutes. The activity was measured viscosimetrically.

Pectinase activity was measured as polymethylgalacturonase (1) with apple pectin (The British Drug Houses Ltd) as substrate in 0.05 M Ac^- buffer, pH 5.0, at 30°C. The method used was the same as for cellulase but with an incubation time of 30 min.

Aryl-β-glucosidase: The ethanol-dissolved substances were added to the enzyme-buffer solution (0.1 M Ac^- buffer, pH 4.1) as above. After centrifugation 3.0 ml of the solution was mixed with 1.0 ml 0.01 M p-nitrophenol-β-d-glucopyranoside (Koch & Light). After 15 min at 40°C two volumes of 10 % Na_2CO_3 was added and $\Delta E_{410\ nm}$ was determined.

Laccase: After centrifugation of the enzyme-inhibitor mixture in 0.05 M citrate-phosphate buffer, pH 4.6, 0.3 ml 0.1 M guaiacol was added. $\Delta E_{480\ nm}$/15 min at 25°C was measured.

Peroxidase: Assays were performed as described for laccase except the pH was 6.0 and 0.1 ml 0.03 M H_2O_2 was added at zero time.

Tyrosinase: Assays were performed as described for laccase except the pH was 6.5 and pyrocatechol was used as substrate.

RESULTS

Acetone extracts of sapwood, reaction zone and sound heartwood of spruce roots generally gave a distribution according to Table 1, when divided into fractions which were soluble in light petroleum, n-butanone and water. The resins are most common in sapwood, while lignans, phenols and glucosides are most frequent in the reaction zone.

Table 1. --Concentrations, in % of fresh wood, of extractives in sapwood, reaction zone and sound heartwood of spruce roots. Acetone extract subdivided into compounds soluble in light petroleum (resins), n-butanone (lignans, phenols, glucosides) and water (glucosides, carbohydrates).

Solvent	Sapwood	React. zone	Heartwood
Acetone	3.0	8.8	2.5
Light petroleum	1.4	1.0	0.9
n-Butanone	0.7	6.5	1.5
Water	0.9	1.3	0.1

Cellulase was found to be the least susceptible enzyme. None of the fractions of sound sapwood or heartwood affected the activity significantly. Weakly oxidized phenolic compounds from the reaction zone had a slight inhibiting effect (10-15 %). Non-enzymatic or laccase-catalyzed oxidation of this fraction increased the inhibition of cellulase to 50-75 % in concentrations corresponding to that of the wood. Hydroxymatairesinol had a weak effect. No inhibition was obtained with 1-conidendrin up to a concentration of 1 %. The results are presented in Table 2.

Pectinase was more susceptible to the lignans and phenols of the reaction zone than was cellulase. The resins from all three wood samples seemed to contain pectinase inhibiting substances. It should be noted that the resins formed a very fine suspension in the reaction medium, that could not be removed by centrifugation. The fact that cellulase and pectinase were analyzed in the same way supports the suggestion that the inhibition of pectinase was significant and not an artifact caused by this problem. Pectinase activity was totally inhibited by pure 1-conidendrin at a concentration of 0.3 %.

The activity of aryl-β-glucosidase was inhibited by both resinous and phenolic substances at very low concentrations and especially by those present in extracts from the reaction zone. However, there also seem to be compounds present in the extracts, which are able to protect the enzyme from total inhibition at higher concentrations of the extract.

Laccase is totally inhibited by the amounts of phenols and lignans present in the n-butanone and water phases of the reaction zone but not by corresponding fractions of the sound sapwood and heartwood or by any

Table 2. -- Effect of extracts from roots of Norway spruce (sapwood (S), reaction zone (R). and heartwood (H)) on fungal cellulase, pectinase, aryl-β-glucosidase and laccase (from Fomes annosus). Activity in % of control. Concentrations of extractives in the reaction medium refer to those of the fresh wood.

Extract	Concentration	Cellulase			Pectinase			Aryl-β-gluc.			Laccase		
		S	R	H	S	R	H	S	R	H	S	R	H
Light petroleum	1	98	95	86	60	60	25				100	90	100
	1/10	100	95	98	85	60	60				100	95	100
n-Butanone	1	90	85	90	95	60	90	21	46	43	95	0	55
	1/10	100	95	100	100	82	98	22	38	24	95	15	100
	1/100							62	30	24	100	75	
	1/1000							95	45	55			
Water	1	95	90	65	97	80	90				100	0	100
	1/10	100	97	100	100	90	95					20	
	1/100											60	

resinous fraction. l-Conidendrin had no effect. In the reaction zone the concentration of hydroxymatairesinol is as high as 4 %. It is initially oxidized by laccase but the reaction products are inhibiting and cause the reaction to stop very soon.

Commercial horse radish peroxidase and tyrosinase were tested in the presence of equivalent concentrations of n-butanone extractives from sapwood and reaction zone. At a concentration of 1 % the latter inhibited peroxidase activity by 75 %, whereas the sapwood phenolics had no significant effect. Tyrosinase was inhibited about 90 % by the reaction zone and 30 % by the sapwood extractives.

DISCUSSION

The results indicate that the four enzymes tested do not react in a similar way on the same kind of extracts from the different wood samples. The extractives of the reaction zone show an effect only against laccase under the conditions used. These compounds are most likely of phenolic character and are therefore oxidized to quinones, which inhibit the oxidase. As no laccase is active in the reaction media of the other enzymes, the phenols are not oxidized to the same degree in these cases.

Some experiments with autoxidized n-butanone fractions from the reaction zone resulted in a much greater inhibition of cellulase and pectinase than shown in the table. Therefore, under natural conditions, with laccase present together with the other enzymes, the higher phenolic content of the reaction zone might cause more inhibition of the wood-decomposing enzymes.

Natural inhibitors of cellulase have been found in many plant families and in various parts of the plant, including leaves, wood, flowers, fruits and seeds, as reported by Mandels & Reese (6). These authors tested extracts of 98 samples of wood against Trichoderma viride cellulase. The inhibitors appeared to be polymerization products of phenols. Simple phenols were inactive (5, 6). Pollard et al (10) found that inhibitors of pectinase were produced from simple phenols by enzyme or autoxidation. Also Grossman (2,3) found that pectinase is unaffected by simple phenols. This indicates that lignans of the reaction zone may partially cause the pectinase inhibition. The effect of resinous substances on this enzyme is remarkable, and, to our knowledge, has not been reported earlier.

Gluconolactone is known to be a specific inhibitor to aryl-β-glucosidase (11). The result obtained in the present study is certainly not caused by such compounds. It may be dependent on stimulating or protecting factors and inhibiting compounds interfering with each other. There might also be glucosides present causing a competitive inhibition.

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UNTERSUCHUNGEN ÜBER DIE PILZHEMMWIRKUNG DES FICHTENHOLZES (PICEA ABIES KARST.)

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PROBLEMSTELLUNG

Nach einer Arbeitshypothese von Laatsch et al. (11) hängt der Befall der Fichte (Picea abies Karst.) durch Kernfäulepilze nicht nur von der Virulenz der Parasiten, sondern auch von der Anfälligkeit der Wirtspflanze ab. Zu den Faktoren, die die Kernfäuledisposition beeinflussen könnten, gehören u.a. die Gehalte an Pilzhemmstoffen im Bast und im Holz der Fichte. Wir gehen davon aus, daß fungistatische Wirkungen der Bast- und Holzgewebe in Abhängigkeit vom Standort und vom Ernährungszustand variieren.

Fomes annosus (Fr.) Cooke, einer der wichtigsten Kernfäuleerreger, kann offensichtlich unter bestimmten Bedingungen unverletzte Fichtenwurzeln befallen. Dabei muß er die Periderme und den Bast der Wurzel durchdringen, um ins Holz zu gelangen (2,3, Schönhar, persönliche Mitteilung). Nun hemmt jedoch der frische Stamm- und Wurzelbast der Fichte im Biotest auf Malzagar das Mycelwachstum von F. annosus (1). Peek et al. (13) konnten elektronenmikroskopisch beobachten, daß die Hyphen von F. annosus im Wachstum gehemmt werden, wenn sie in die Phlobaphensubstanz des Bastes eindringen. Diese Pilzhemmung beruht zum größten

Teil auf dem Gehalt des Bastes an freien Polyhydroxyphenolen (Stilbenderivaten und flavonoiden Verbindungen) und ihren Glucosiden, zum geringeren Teil auf Harzen und Harzderivaten. Der fungistatische Effekt hängt darüber hinaus ab von den im Bast vorhandenen pilzfördernden Stoffen (z.B. Kohlenhydraten und Aminosäuren). Er wird durch Wassermangel, Manganmangel und durch eine Überdüngung mit Stickstoff zumindest zeitweilig herabgesetzt (1). Es ist indessen noch ungeklärt, ob solche standorts- und ernährungsbedingten Variationen der Hemmwirkung des Fichtenbastes für das Eindringen von F. annosus in intakte Wurzeln unter Freilandbedingungen relevant sind.

Aus den Untersuchungen von Shain (17) und Shain und Hillis (18) sowie Diaz-Palacio (7) ist außerdem bekannt, daß auch im Stammholz der Fichte chemische Abwehrmechanismen existieren, die die Ausbreitung von F. annosus im Splint nach erfolgreichem Durchdringen des Bastes oder nach einem Befall über Wunden und Wurzelverwachsungen behindern könnten. Gelegentlich beobachten wir eine verstärkte Harzbildung. An der Befallsgrenze zwischen dem intakten Splint und dem befallenen Reifholz tritt zudem häufig eine sogenannte Reaktionszone (17) auf. Sie zeichnet sich aus durch totes, fast stärkefreies Parenchym, hohe Kalium-, Calcium- und Magnesiumgehalte, alkalische Reaktion, hohe Lignankonzentrationen und eine deutliche Hemmwirkung gegenüber F. annosus im Biotest (7, 17, 18). Der fungistatische Effekt dieser Zone, die als unspezifische Reaktion des Splintholzes auf den Pilzangriff während des Absterbens der Parenchymzellen gebildet werden dürfte, soll vorwiegend auf einer Anreicherung von Hydroxymatairesinol beruhen (18).

Das Reifholz der Fichte kann zwar im Gegensatz zum Splint keine Abwehrreaktionen durchführen, weil es sich um totes Gewebe handelt. Aus den Untersuchungen von Freudenberg und Knof (8) geht jedoch hervor, daß auch das Reifholz gesunder Fichten Lignane enthält, die teilweise Hemmstoffcharakter besitzen dürften.

Ausgehend von diesen Befunden interessierten wir uns dafür, ob auch gesundes Fichtenreifholz fungistatisch wirken kann, und ob die Pilzhemmung dieses Reifholzes und der Reaktionszonen im Stamm fauler Fichten vom Standort abhängt. Um erste Anhaltspunkte zu gewinnen, untersuchten wir deshalb Holzproben, die aus unterschiedlich fäulegefährdeten Beständen der Münchner Umgebung stammten, auf ihre fungistatischen Effekte und auf mehrere holzchemische Eigenschaften.

MATERIAL

Zu diesem Zweck wählten wir in zwei rund 60-jährigen Fichtenbeständen auf verschiedenen Bodenformen gesunde und faule Fichten aus. Der eine Bestand stockt in 500 m Seehöhe

auf einer verlehmten Pararendzina (örtlich Parabraunerde) aus Niederterrassenschotter im Forstamt München-Nord und ist weitgehend frei von Kernfäule; die wenigen kranken Bäume waren durchweg wundfaul, die Erreger der Fäule wurden nicht bestimmt. Bei dem Vergleichsstandort handelt es sich um eine Kalkpaternia aus den Isarauen (450 m) im Forstamt Freising, auf der die Fichten stark kernfäulegefährdet sind. Abimpfungen ergaben, daß hier ausschließlich F. annosus als Kernfäuleparasit auftritt. Großklimatisch sind beide Standorte weitgehend vergleichbar. Die mittlere Jahrestemperatur liegt bei 7,2 - 7,5 °C; die Jahresniederschläge variieren zwischen 700 - 850 mm.

Insgesamt gewannen wir im Winter von 5 gesunden und 6 faulen Fichten Stammabschnitte aus vergleichbarer Baumposition (30-60 cm über dem Boden). Sie wurden nach Farbmerkmalen des frischen Querschnitts oder nach Besprühen mit spezifischen Reagenzien wie folgt in verschiedene Holzzonen geteilt:

a) Gesunde Stammabschnitte, die am Abhieb frei von Fäulemerkmalen waren, trennten wir in Splint und Reifholz. Letzteres färbte sich nach Besprühen mit dem Phenolindikator Diazosulfanylsäure in charakteristischer Weise rötlich.

b) Bei faulen Bäumen war zwischen zwei Fällen zu unterscheiden:

1. Die Fäule beschränkte sich auf den zentralen Teil des Reifholzes oder auf einen Sektor des Stammquerschnitts. In diesen Fällen war zwischen Faulholz und Splint noch intaktes Reifholz vorhanden, das mit Diazosulfanylsäure die charakteristische Rotfärbung zeigte. Das faule Reifholz konnte weiter zerlegt werden in Partien mit einer initialen Fäule, die sich häufig nur in einer Verfärbung und beginnender Zersetzung äußerte, und in Zonen einer fortgeschrittenen Fäule, deren Holz bereits intensiv zersetzt war. In beiden Fäulestadien waren parasitische Fäulepilze nachweisbar.

2. Die Fäule war bis zum Splint vorgedrungen. In diesem Fall war zwischen Splint und faulem Reifholz regelmäßig eine Reaktionszone ausgebildet. Wir teilten sie in eine innere grüne Zone, die an das Faulholz angrenzte, und einen äußeren weißen Abschnitt, der in Kontakt mit dem frischen Splint stand. Beide Zonen waren frei von Pilzhyphen und reagierten nach Besprühen mit dem pH-Indikator Bromkresolgrün alkalisch. Sowohl die grüne als auch die weiße Zone waren offensichtlich phenolreicher als das angrenzende Splintholz, denn mit Diazosulfanylsäure ergaben sie die charakteristische Rotfärbung.

Abb. 1 informiert in schematischer Darstellung über die durchgeführte Trennung und die räumliche Anordnung der verschiedenen Holzzonen auf dem Stammquerschnitt der

Probebäume. Die dort angegebenen Symbole für die Holzzonen werden auch in den weiteren Abbildungen beibehalten.

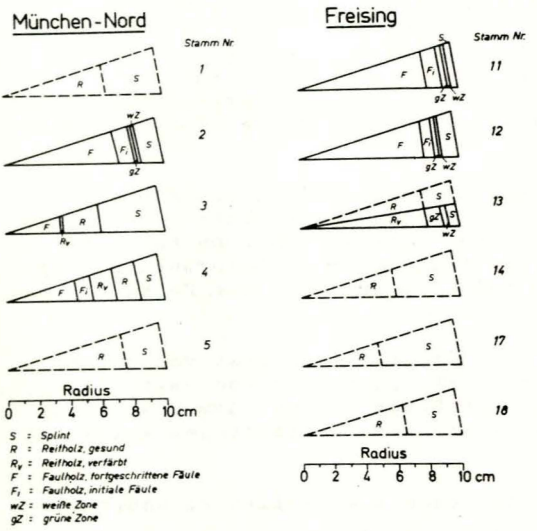

Abb. 1 Schematische Darstellung über die Anordnung der Holzzonen in den untersuchten Bäumen.

Schematic representation of the wood zones in the examined trees.

----- gesunde Stämme (healthy stems)

_____ befallene Stämme (infected stems)

S = sapwood, R = healthy heartwood, R_y = stained heartwood F = heavily decayed wood, F_i = initial stage of decayed wood, wZ = white zone, gZ = green zone.

METHODIK

Aus sämtlichen Holzzonen gewannen wir Proben, die bei - 20 °C aufbewahrt und vor der Verarbeitung fein gemahlen wurden (Korngröße < 0,5 mm). Durch Trockeneiszusatz beim Mahlvorgang wurden Oxydationen verhindert!

Hemmwirkung des Holzmehls im Biotest

Die Prüfung des Holzmehls auf fungistatische Wirkung erfolgte in Anlehnung an Wenzel et al. (20) auf Malzextraktagar (2 % Agar-Agar; 4 % Malzextrakt; 1 g Holzmehl je 20 ml Agar). Alle Testserien führten wir sowohl in Petrischalen (20 ml Agar) als auch in flach ausgelegten Reagenzröhrchen (3 ml) durch. Hierzu wurde Malzextraktagar zusammen mit dem darin homogen verteilten Holzmehl autoklaviert (121 °C; 20

min) und nach Erstarren des Agars mit frischem F. annosus-Mycel beimpft (Durchmesser des Impfstückes = 5 mm).

Nach einer Inkubationszeit von 6-7 Tagen bei 23 °C bestimmten wir in den Schalen die radiale, in den Röhrchen die longitudinale Ausdehnung des Mycels, wobei für die Kontrollen Malzextraktagar ohne Holzmehlzusatz benützt wurde. Als Maß für die fungistatische Wirkung verwendeten wir die relative Hemmwirkung (HW), die sich nach folgender Formel errechnet:

$$HW\ (\%) = \left[1 - \frac{\text{Mycelausdehnung der Testprobe}}{\text{Mycelausdehnung der Kontrolle}}\right] \times 100$$

Hemmwirkung der Methanolextrakte

Außerdem wurde auch die Fraktion der methanollöslichen Stoffe des Fichtenholzes im Röhrchentest auf ihre fungistatische Wirkung untersucht. Zu diesem Zweck versetzten wir Malzagar mit Methanolextrakten in den Konzentrationen 0,2; 0,5 und 1,0 %. Die Methanolextrakte verteilten sich in flüssigem Agar nur schlecht und mußten deshalb zuvor in 80%igem Äthanol gelöst werden. Die Konzentration des Äthanols in Agar lag bei 2 %. Da Äthanol in dieser Konzentration das Mycelwachstum von F. annosus deutlich hemmte, erhielten auch die Kontrollen (ohne Methanolextrakt) den gleichen Äthanolzusatz.

Physikalische und chemische Analysen

Für die Bestimmung der Rohdichte wurden aus den verschiedenen Holzzonen jeweils mehrere Proben bei 105 °C getrocknet und gewogen. Wir ermittelten ihr Volumen mit dem Volumenmesser nach Breuil.

Die Feuchtigkeit des gemahlenen Materials bestimmten wir nach Trocknung bei 105 °C.

Für die Bestimmung von P, K, Ca, Mg, Fe und Mn wurden 3 g frisches Holzmehl bei 450 °C verascht, mit 5 ml heißer 10 %iger HCl aufgenommen und auf 50 ml aufgefüllt.

Aus dieser Lösung wurde P photometrisch mit Ammonium-Vanadat und Ammonium-Molybdat, K und Ca flammenphotometrisch, Mg atomabsorptionsspektrophotometrisch, Fe photometrisch mit Orthophenanthrolin und Mn photometrisch als K-Permanganat analysiert. Die Bestimmung des Gesamtstickstoffs erfolgte nach Kjeldahl, diejenige von B photometrisch mit 1,1' - Dianthrimid-Schwefelsäure.

Zur Gewinnung der harzartigen Bestandteile extrahierten wir das frische Holzmehl unter Rückfluß quantitativ mit Petroläther. Nach Verdampfung des Lösungsmittels verblieb als gelblich-dickflüssiger Rückstand der Petrolätherextrakt.

Das mit Petroläther extrahierte Holzmehl wurde anschließend mit Methanol in CO_2-Atmosphäre erschöpfend extrahiert, das Lösungsmittel im Vakuum eingedampft. Wir erhielten auf diese Weise den Methanolextrakt in fester, schaumartiger Form.

Chromatogramme des Methanolextrakts wurden zweidimensional auf Kieselgeldünnschichtplatten (Selecta G 1500) durchgeführt. Als Laufmittel verwendeten wir Benzol-Aceton (6 : 4) und Chloroform-Essigsäure (8 : 2). Die Lignane wurden nach Besprühen mit Phenolreagenzien wie z.B. Folin-Ciocalteus Reagenz oder Diazosulfanylsäure sichtbar.

Die Konzentration und Zusammensetzung der Aminosäuren im Methanolextrakt wurde chromatographisch auf Celluloseplatten (Selecta G 1440) ermittelt. In der ersten Laufrichtung benutzten wir Chloroform-Methanol-17proz.Ammoniaklösung (4 : 4 : 2), in der zweiten Richtung Phenol-Wasser (7,5 : 2,5 g/g, Gasphase äquilibriert mit 3proz.Ammoniaklösung). Wir entwickelten die Chromatogramme mit Ninhydrin-Reagenz (4).

Die im Methanolextrakt enthaltenen Zucker wurden chromatographisch nach De Stefanis und Ponte (6) auf Kieselgelplatten mit Chloroform-Essigsäure-Wasser (3,0 : 3,5 : 0,5) getrennt und nach Besprühen mit Diphenylamin-Anilin identifiziert.

Um die Gesamtmenge an Kohlenhydraten im Splint aus den verschiedenen Bäumen zu bestimmen, wurden je 0,5 g gemahlenes Material mit 25 ml Wasser 24 Std. lang ausgeschüttelt. Die Lösungen zentrifugierten wir, versetzten sie mit Aktivkohle und filtrierten sie durch eine Glasfritte. In den klaren Lösungen bestimmten wir die Zucker kolorimetrisch mit Anthronreagenz (12) gegen Standardlösungen von Glucose.

Die Gesamtmenge an Lignanen im Methanolextrakt - bezogen auf Conidendrin - wurde wie folgt bestimmt: Wir maßen die optische Dichte von 5 mg des jeweiligen Methanolextrakts gelöst in 100 ml absolutem Äthanol bei 282 nm. Mit Hilfe des molaren Extinktionskoeffizienten für Conidendrin ($\varepsilon = 7{,}2 \times 10^3$) (9) konnte die darin enthaltene Menge an Lignanen berechnet werden.

ERGEBNISSE

Pilzhemmwirkung des Fichtenholzes

Fichtenholzproben, die aus verschiedenen Beständen, von gesunden und befallenen Bäumen und aus unterschiedlichen Positionen des Baumquerschnitts stammten, wichen in ihrer Hemmwirkung auf das Mycelwachstum von F. annosus im Biotest deutlich voneinander ab (Tab. 1).

Tab. 1 Relative Hemmwirkung auf Fomes annosus (%)
Relative inhibition of the wood samples of Fomes annosus (%)

Standort	Stamm-Nr.		S	wZ	gZ	R	R_v	F_i	F
	1)	gesund	13,0			n.b.			
	5)		24,0			42,0			
München-Nord									
	2)		4,5	24,0	n.b.			21,5	23,0
	3)	krank	- 0,8			32,0	n.b.		27,0
	4)		1,6			55,0	57,0	23,0	22,0
	14)		5,0			14,0			
	17)	gesund	9,2			12,5			
	18)		1,0			17,2			
Freising									
	11)		8,3	n.b.	25,0			10,0	20,5
	12)	krank	6,5	n.b.	31,5			29,0	21,0
	13)		14,5	26,5	24,0	19,5	17,0		

n.b. = nicht bestimmt

Splintproben hemmten in der Regel nur geringfügig. Eine Zugabe von Holzmehl aus dem Reifholz sowie aus den weißen und grünen Abschnitten der Reaktionszone beeinträchtigte dagegen das Mycelwachstum auf Malzextraktagar erheblich. Dabei hemmten die Reifholzproben von gesunden Bäumen des Bestandes München-Nord signifikant stärker (Mittelwert = 43 %) als vergleichbare Reifhölzer von Freising (16 %). Unter den Freisinger Proben ging die größte Hemmwirkung von den Reaktionszonen aus (27 %), die somit eine chemische Barriere für die Ausbreitung des Pilzes im Stamm darstellen dürften. Substrate mit Holzmehlzusatz aus Reifhölzern und Reaktionszonen zeigten am Ende der Inkubationszeit eine Braunfärbung. Diese ist auf den Gehalt des Holzes an phenolischen Verbindungen (v.a. Lignanen) zurückzuführen, die durch Pilzenzyme (Laccasen) oxydiert werden und polymerisieren.

Mikroskopische Beobachtungen

Längs- und Querschnitte aus den weißen und grünen Teilen der Reaktionszone zeigten oftmals eine auffallende Anreicherung von kristallinen Einschlüssen in den Tracheidenlumina (Abb. 2b). Vielfach traten diese Kristalle in den spitz zulaufenden Faserendigungen gehäuft auf (Abb. 2a).Die Kristalle waren teils kubisch, hexagonal, rhombisch oder monoklin, teils nadel- bis sternförmig und lösten sich in verdünnter Salzsäure. Es dürfte sich um Calciumcarbonatkristalle handeln. Daneben traten in den Markstrahlparenchymzellen, aber auch in den Tracheiden, amorphe Einschlüsse auf (Abb.3), die sich in Methanol lösten und die möglicherweise Lignane darstellten.

Physikalische und chemische Eigenschaften

Rohdichte: Die Rohdichte der Holzproben (Trockengewicht/Trockenvolumen) variierte nur wenig. Die Mittelwerte für Splintholz lagen bei 0,50 g/cm^3 Trockensubstanz, für Reifholz bei 0,45 g/cm^3. Proben aus den grünen und weißen Zonen erreichten geringfügig höhere Werte bis 0,60 g/cm^3. Ihre höhere Dichte ist möglicherweise auf die mikroskopisch nachgewiesenen Einlagerungen zurückzuführen.

Feuchtigkeit: Die Wassergehalte schwankten ohne signifikante Unterschiede zwischen den beiden Standorten für das Splintholz zwischen 100 und 140 %, für das Reifholz zwischen 20 bis 40 % der Trockensubstanz (Abb. 4). Die Reaktionszonen enthielten in den weißen Abschnitten ebenfalls 30 - 40 %, in den grünen Teilen jedoch 60 - 70 % Wasser.

Stickstoff: Die N-Gehalte im Splint und Kern gesunder Fichten lagen sowohl in München-Nord als auch in Freising auf gleichem Niveau bei etwa 500 ppm (Abb. 5). Auffallend ist, daß einige faule Fichten auf beiden Standorten vielfach über den ganzen Stammquerschnitt hinweg mehr Stickstoff enthielten als ihre gesunden Nachbarn.

Phosphor: Die Phosphorgehalte schwankten in den untersuchten Holzproben von 10 bis 150 ppm. Splintholz war im allgemeinen etwas P-reicher als Reifholz. Unterschiede zwischen den Standorten sowie zwischen gesunden und faulen Bäumen waren nicht erkennbar (Tab. 2).

Kalium: Im Splint und Kern gesunder Bäume variierten die Kaliumkonzentrationen auf beiden Standorten zwischen 150 und 350 ppm (Abb. 6). Während die infizierten Bäume im intakten Splint nur geringfügig mehr K enthielten, war Kalium in der Reaktionszone und im befallenen Reifholz meist erheblich angereichert. Durch Berechnung der Kaliummengen auf Volumenbasis konnten wir nachweisen, daß es sich dabei um eine absolute K-Akkumulation und nicht etwa um einen Konzentrationseffekt handelte (15, 17, 19).

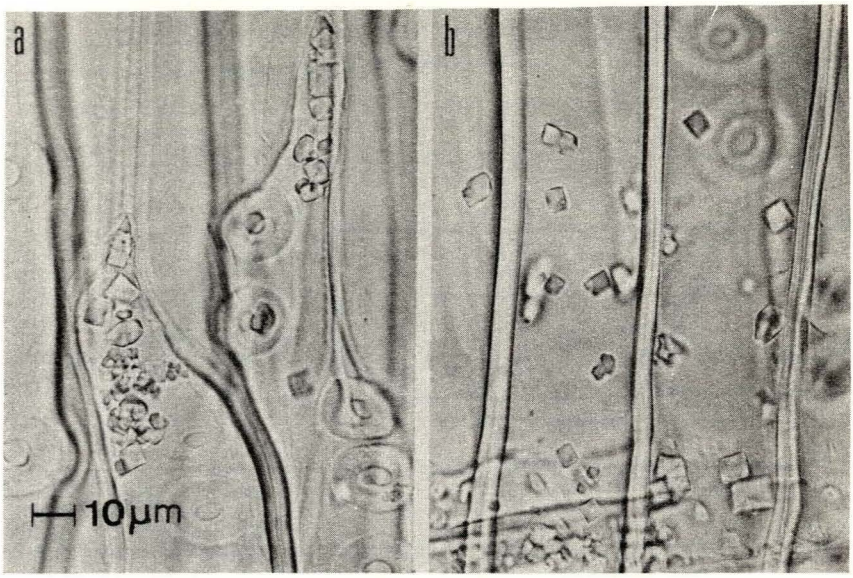

Abb. 2 Kristalline Einschlüsse in den Tracheiden aus der Reaktionszone einer Fomes annosus-befallenen Fichte. Crystalline inclusions in tracheids from the reaction zone of a spruce infected by Fomes annosus

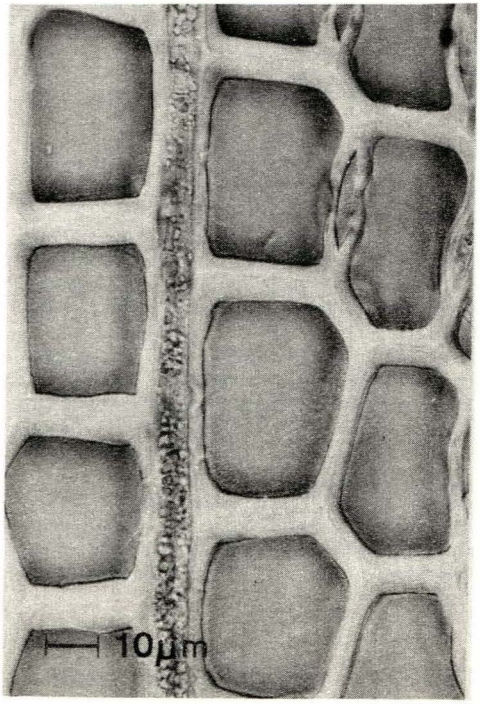

Abb. 3 Amorphe Einschlüsse in einem Markstrahl aus der Reaktionszone einer Fomes annosus-befallenen Fichte. Amorphous inclusions in wood ray cells from the reaction zone of a spruce infected by Fomes annosus

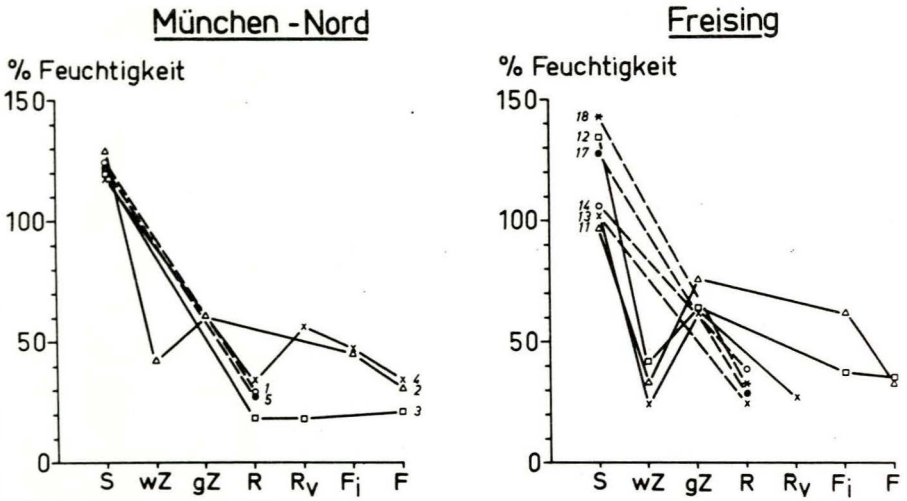

Abb. 4 Wassergehalte der Holzproben bezogen auf Trockensubstanz
Moisture content of the wood samples related to dry weight

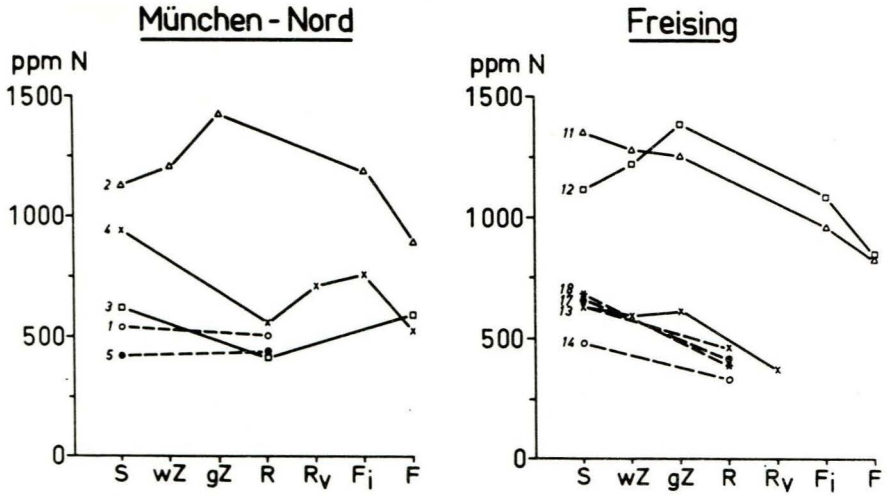

Abb. 5 Stickstoffgehalte der Holzproben
Nitrogen content of the wood samples

Tab. 2 Phosphor- und Mangangehalte der Holzproben
Amount of phosphorus and manganese in the wood samples

Standort	Ges.-zustand	S	wZ	gZ	R	R_v	F_i	F
					ppm P			
München - Nord	gesund	122-128			62- 64			
	krank	51-144	78	63	13- 46	27- 50	62-77	19- 72
Freising	gesund	7- 47			1- 9			
	krank	123-124	45-114	59-91	n.b.	n.b.	36-66	71-103
					ppm Mn			
München -Nord	gesund	90			62- 66			
	krank	57- 75	112	318	74-105	92-127	131	80
Freising	gesund	+			+			
	krank	+	+	+	n.b.	n.b.	+	+

n.b. = nicht bestimmt
+ = Spuren

Auch der Baum Freising Nr. 13, bei dem aus derselben Stammscheibe ein gesunder und ein befallener Sektor untersucht wurde, zeigte genau die gleiche Differenzierung in der Kaliumverteilung, und nur der schwach befallene Baum München-Nord Nr. 3 wich von dieser allgemeinen Tendenz ab.

Calcium: Eine prinzipiell ähnliche Verteilung fanden wir für Calcium (Abb. 7). Die Ca-Gehalte im Splintholz schwankten auf beiden Standorten sowohl bei gesunden als auch bei kranken Bäumen zwischen 500 und 1000 ppm, im Reifholz von 800 bis 1200 ppm. Weiße Zone, grüne Zone, verfärbtes Kernholz und Reifholz mit initialer Fäule waren - ausgenommen Baum Freising Nr. 13 - erheblich Ca-reicher. Die Calciummaxima wurden bei allen Fichten, die eine Reaktionszone besaßen, immer in deren grünem Abschnitt erreicht. Diese Spitzenwerte lagen auf dem carbonatreichen Standort Freising bedeutend höher als in München-Nord. Das stark zersetzte Faulholz im zentralen Teil der Stammscheiben unterschied sich in den meisten Fällen im Calciumgehalt nicht vom intakten Reifholz gesunder Nachbarn.

Magnesium: Die Magnesiumkonzentrationen in den Stammscheiben folgten fast genau den gleichen Gradienten wie Calcium (Abb. 8). Die Werte lagen im Splint zwischen 50 und 100 ppm unabhängig davon, ob es sich um gesunde oder faule

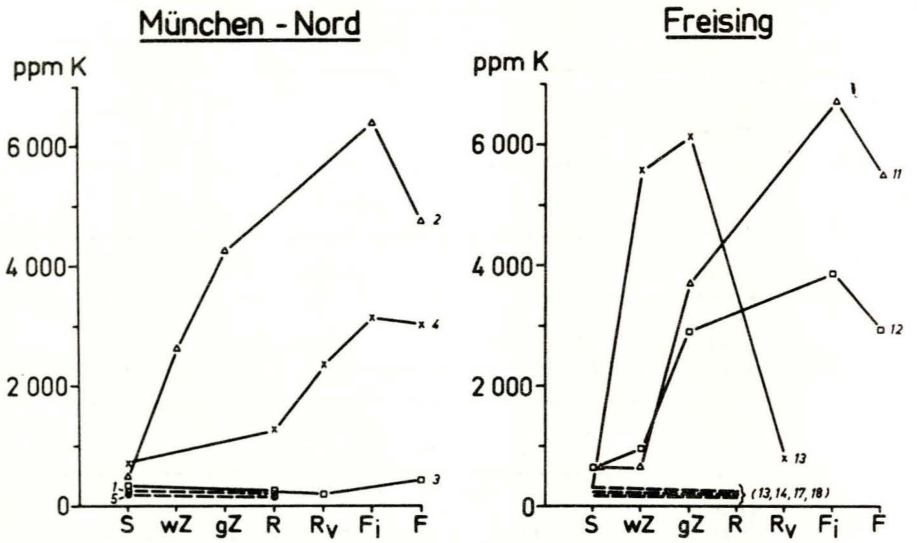

Abb. 6 Kaliumgehalte der Holzproben
Potassium content of the wood samples

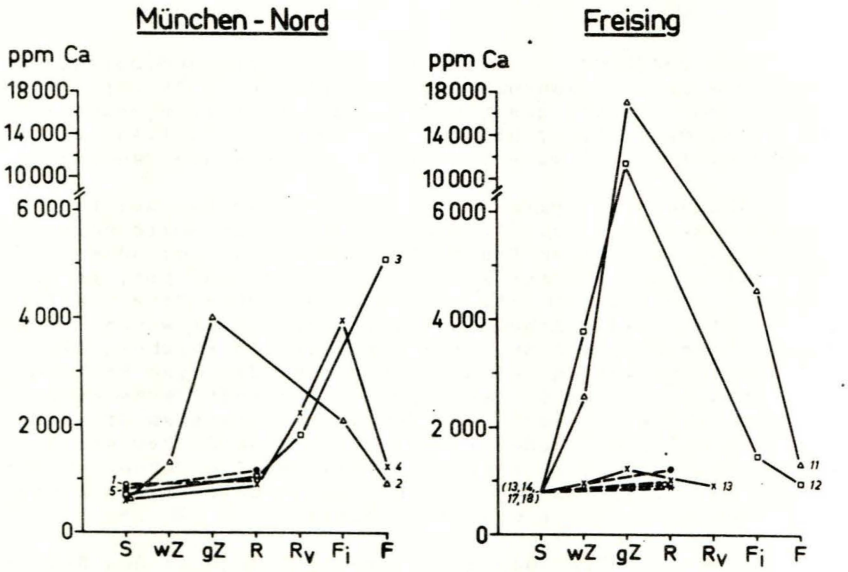

Abb. 7 Calciumgehalte der Holzproben
Calcium content of the wood samples

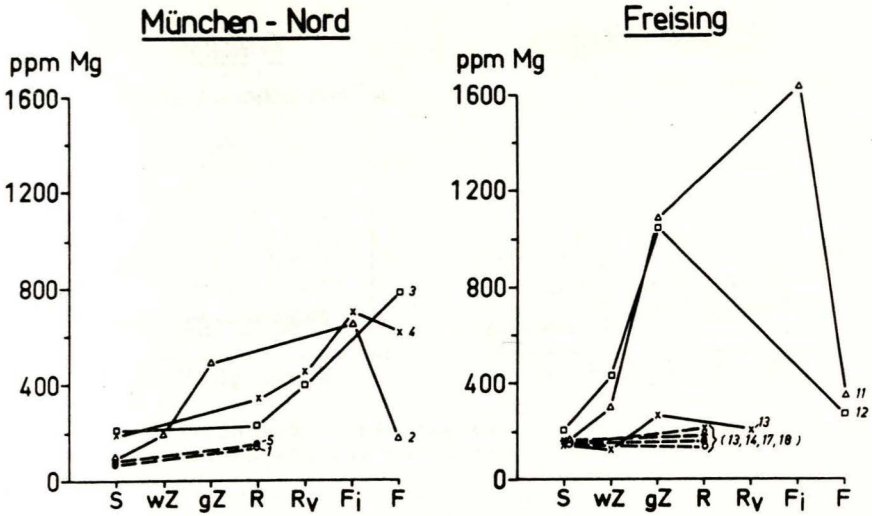

Abb. 8 Magnesiumgehalte der Holzproben
Magnesium content of the wood samples

Bäume handelte. Gesundes Kernholz erreichte in der Regel etwas höhere Werte. Reaktionszonen und befallene Reifhölzer zeichneten sich indessen durch einen kräftigen Anstieg der Mg-Gehalte aus. Stark zersetztes Faulholz enthielt dagegen häufig wieder weniger Magnesium.

Mangan: Während in München-Nord Splint und Reifholz der Fichten zwischen 50 und 100 ppm Mangan enthielten (Tab. 2), und in der grünen Zone des stark befallenen Baumes Nr. 2 sogar 300 ppm erreicht wurden, konnten wir in sämtlichen Holzproben aus Freising höchstens Manganspuren nachweisen (5).

Eisen und Bor: Die Gehalte an diesen Elementen variierten bei allen Probebäumen über den gesamten Stammquerschnitt hinweg fast auf dem gleichen Niveau zwischen 9 und 74 ppm Fe sowie 1 und 6 ppm B.

Petrolätherextrakte: Bestimmte Komponenten des Fichtenharzes, wie z.B. die Abietinsäure, wirken auf F. annosus hemmend (17). Dieser Gesichtspunkt bestimmte uns, die Holzproben auch auf ihren Gehalt an in Petroläther löslichen Stoffen zu untersuchen. Die Petrolätherextrakte enthalten allerdings neben Harzsäuren auch Fette, Kohlenwasserstoffe, Wachse, höhere Alkohole und Glyceride (10). Bei den meisten Bäumen lieferte der Splint etwas größere Extraktmengen als das gesunde oder befallene Reifholz. Die Konzentrationen lagen im Splint der Fichten in München-Nord zwischen 0,8 und 1,6 %, im Kern zwischen 0,5 und 1,2 % ohne Unterschied zwischen gesunden und kranken Bäumen (Abb. 9). Die gesunden Freisinger Fichten enthielten im Splint 0,8 - 1,0 %, im

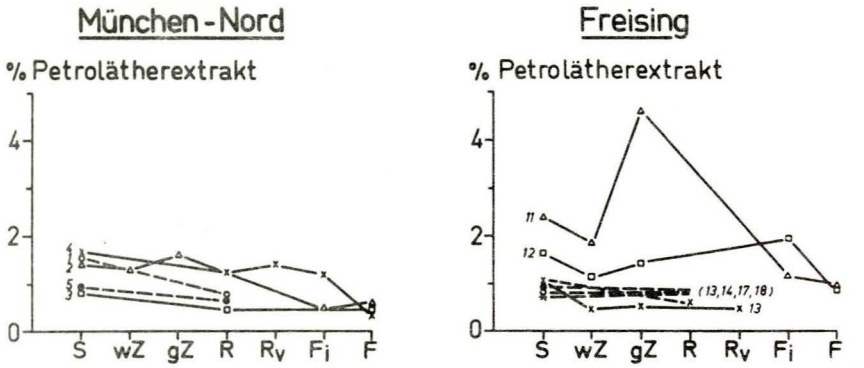

Abb. 9 Gehalte an Petrolätherextrakt in den Holzproben
Content of light petroleum soluble extractives of the wood samples

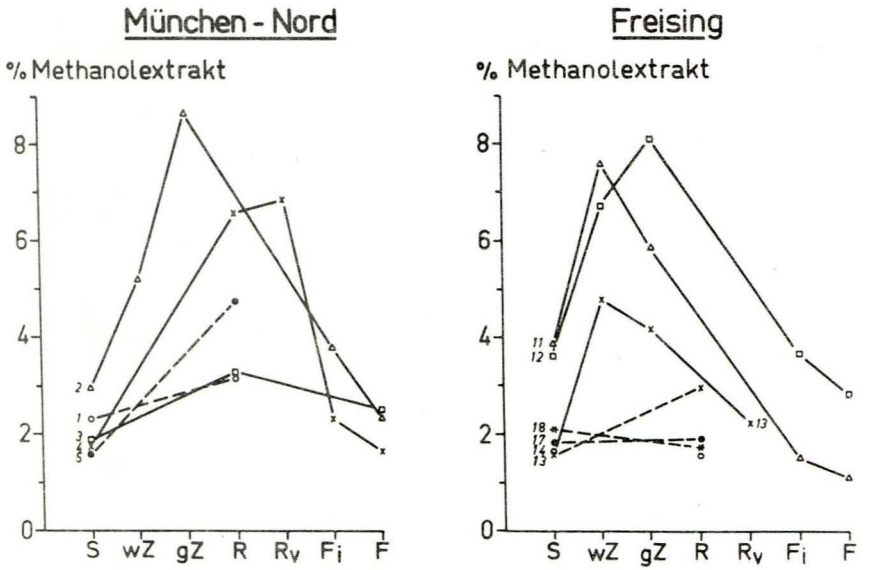

Abb. 10 Gehalte an Methanolextrakt in den Holzproben
Content of methanol soluble extractives of the wood samples

Reifholz 0,8 - 0,9 % Petrolätherextrakt. Hier heben sich allerdings die stark befallenen Fichten Nr. 11 und 12 durch deutlich höhere Gehalte an petrolätherlöslichen Stoffen von den übrigen Probebäumen ab. Bei beiden Fichten hatte die Fäule am Stammfuß den Splint bereits an einzelnen Stellen durchbrochen und einen kräftigen Harzfluß auf der Rinde ausgelöst.

Methanolextrakte: Die methanollösliche Fraktion des Splintholzes ist ein sehr heterogenes Stoffgemisch, das sich hauptsächlich aus Lignanen, freien Zuckern und Aminosäuren zusammensetzt. Sein Anteil schwankte auf beiden Standorten von 1,5 - 3,8 % der Trockensubstanz (Abb. 10).

Im Reifholz dagegen besteht der Methanolextrakt überwiegend aus Lignanen, deren Struktur von Freudenberg und Knof (8) aufgeklärt wurde.

Die gesunden Fichten von den beiden überprüften Standorten unterschieden sich im Gehalt ihres Reifholzes an Methanolextrakt erheblich. Die Bäume von München-Nord, wo die Fichten durch Kernfäule kaum bedroht sind, enthielten im Reifholz 3 - 6 % Methanolextrakt, die Vergleichsfichten in Freising erreichten dagegen nur 1,5 - 3 %. Während die Methanolextraktgehalte im faulen Reifholz der Freisinger Bäume im Variationsbereich der gesunden Reifhölzer lagen, zeichneten sich weiße und grüne Reaktionszonen durch signifikant höhere Werte zwischen 4 und 8 % aus. Auch die grüne Zone des Baumes Nr. 2 von München-Nord enthielt über 8 % methanollösliche Stoffe.

Zusammensetzung der Methanolextrakte:

Lignane: Die UV-Spektren der Methanolextrakte zeigten den für Lignane typischen Verlauf mit einem Absorptionsmaximum bei 282 nm. Der Anteil der Lignane (bezogen auf Conidendrin) im Methanolextrakt lag bei allen Reifhölzern sowie bei den weißen und grünen Zonen zwischen 60 und 70 %. Von der methanollöslichen Fraktion des Splintholzes entfielen dagegen in München-Nord durchschnittlich 36 %, in Freising sogar nur 20 % auf Lignane.

Abb. 11 zeigt das Dünnschichtchromatogramm eines repräsentativen Methanolextrakts aus Fichtenreifholz. Unter den Lignanen dominierte in allen Holzzonen Hydroxymatairesinol. Daneben traten als wichtige Nebenbestandteile u.a. Allohydroxymatairesinol, Matairesinol und Conidendrin auf.

Aminosäuren: Durch Dünnschichtchromatographie der Methanolextrakte konnten wir zeigen, daß die Bäume von Freising im Splint etwa fünfmal mehr freie Aminosäuren enthielten als die Bäume von München-Nord. Die Hauptkomponenten waren Threonin, Alanin, β-Alanin, Asparaginsäure und Arginin.

Abb. 11 Dünnschicht-Chromatogramm des Methanolextrakts aus Fichtenreifholz. Kieselgel. Fließmittel: Benzol-Aceton (6 : 4) und Chloroform-Essigsäure (8 : 2). Besprühmittel: Folin-Ciocalteus Reagenz.
Thin-layer chromatogram of the methanol extract from Norway spruce heartwood. Silica gel. Solvents: benzene-acetone (6 : 4) and chloroform-acetic acid (8 : 2). Spray reagent: Folin-Ciocalteus

Kohlenhydrate: Der Splint der nicht infizierten Freisinger Fichten enthielt mehr Kohlenhydrate (4,2 mg/g Tr.S.) als das Splintholz gesunder Bäume aus dem Bestand München-Nord (0,8 mg/g Tr.S.). Auf beiden Standorten übertrafen die faulen Fichten im Zuckergehalt ihres Splints die gesunden Nachbarn beträchtlich (10,7 mg/g Tr.S. in Freising bzw. 4,3 mg/g Tr.S. in München-Nord). Als wichtigste Zucker waren chromatographisch Glucose und Fructose nachzuweisen.

Beziehungen zwischen der Pilzhemmwirkung und einigen chemischen Holzkomponenten

Wir prüften mit Hilfe multipler Regressionsanalysen, welche statistischen Abhängigkeiten zwischen der Pilzhemmwirkung der verschiedenen Holzzonen im Biotest und ihrem Gehalt an einigen chemischen Komponenten vorliegen (Tab. 3). Von dieser Analyse waren sämtliche faulen, von den Pilzen besiedelten Holzproben ausgeschlossen, weil ihre stoffliche Zusammensetzung sicher durch die Pilze bereits verändert war.

Tab. 3 Beziehungen zwischen der relativen Hemmwirkung und einigen chemischen Holzkomponenten.
Ergebnisse multipler linearer Regressionsanalysen
Programm BMD O2 R
Relationship between the relative inhibition and some chemical wood components.
Results of multiple linear regression analysis
programm BMD O2 R

x-Variable	München-Nord (n = 9) r	Signifikanz von r	Freising (n = 15) r	Signifikanz von r
H_2O (%)	- 0,85	++	- 0,59	+
N (ppm)	0,13	n.s.	0,57	+
K (ppm)	0,37	n.s.	0,73	++
Ca (ppm)	0,76	+	0,61	+
Mg (ppm)	0,74	+	0,70	++
log (Petrol-ätherextrakt %)	0,29	n.s.	0,28	n.s.
log (Methanol-extrakt %)	0,86	++	0,88	+++
log (Lignane %)	0,89	+++	0,92	+++
Multiple Bestimmtheit (%).	87		91	
Variable mit signifikantem Bestimmungsgewicht in der Mehrfachregression.	Lignane		Lignane Methanol-extrakt	
Standardfehler der Schätzung (%).	9,4		3,5	

r = einfache Korrelationskoeffizienten
+++ = P $<$ 0,001
++ = P $<$ 0,01
+ = P $<$ 0,05
n.s.= nicht signifikant

Die Ergebnisse stimmen für München-Nord und Freising darin überein, daß die fungistatischen Effekte der Holzproben mit ihren Gehalten an Methanolextrakt und an Lignanen hochsignifikant positiv korrelierten. In beiden Fällen waren die Lignankonzentrationen besonders straff mit der Pilzhemmung gekoppelt und besaßen auch in den Mehrfachregressionen ein hohes Bestimmungsgewicht. Auch ging die relativ große Hemmwirkung der Reifhölzer (München-N) und Reaktionszonen (Freising) in der Regel mit geringen Wassergehalten einher. In Freising hemmten die K-, Ca- und Mg-reichen Reaktionszonen stärker als die Splinthölzer und gesun-

den Reifholzproben mit geringerem K-, Ca- und Mg-Gehalt, was sich ebenfalls in vergleichsweise hohen Korrelationskoeffizienten widerspiegelt.

Wir vermuten jedoch, daß die Hemmwirkung des Holzes kausal hauptsächlich durch seinen Gehalt an Lignanen bestimmt wird. Diese Hypothese wurde durch Faktorenanalysen gestützt und deckt sich im übrigen mit den Beobachtungen von Shain und Hillis (18) sowie Diaz-Palacio (7).

Die Regressionslinien für den Zusammenhang zwischen der Hemmwirkung und dem Lignangehalt der Holzproben weichen für die beiden untersuchten Bestände voneinander ab; denn die Steigung der Ausgleichsgeraden ist für München-Nord größer als für Freising (Abb. 12). Qualitative chromatogra-

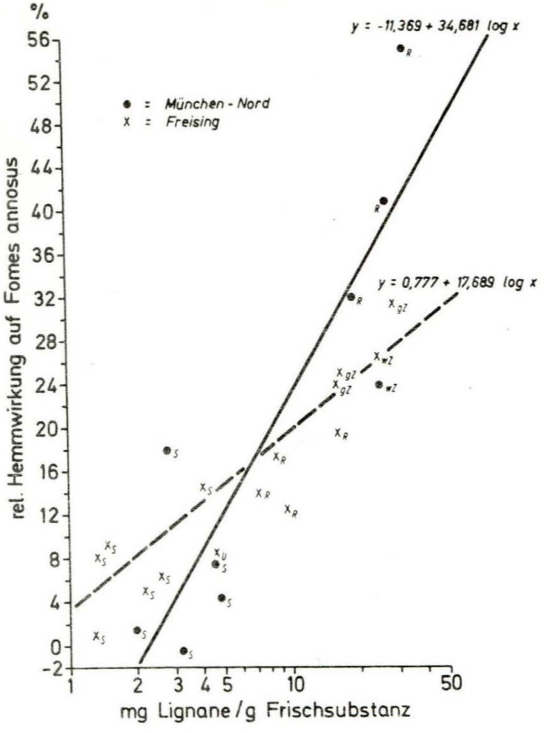

Abb. 12 Beziehungen zwischen der relativen Hemmwirkung (%/g Frischsubstanz) und der Lignankonzentration der Holzproben
Relationship between the relative inhibition (%/g fresh matter) and the lignan concentration of the wood samples

phische Analysen weisen darauf hin, daß diese Differenz auf einer verschiedenartigen Zusammensetzung der Lignanfraktion

beruhen könnte.

Die Methanolextrakte aus dem Reifholz einiger Probebäume beeinträchtigten im Konzentrationsbereich 0,2 - 1,0 % das Mycelwachstum von Fomes annosus auf Agar ebenfalls erheblich (relative Hemmung der 1 %igen Methanolextrakte aus dem Reifholz von München-Nord 40 - 47 %; aus der grünen und weißen Zone 32 - 36 %). Die fungistatische Wirkung des Holzmehls ließ sich in diesen Fällen mit befriedigender Genauigkeit auf Menge und Hemmwirkung ihrer methanollöslichen Fraktion zurückführen. Abweichungen in der Pilzhemmwirkung, die zwischen verschiedenen Methanolextrakten auftraten, waren stets durch den wechselnden Lignananteil zu erklären.

DISKUSSION

Rennerfelt und Nacht (14) hatten im Fichtenreifholz keine fungitoxischen Stoffe nachweisen können. Shain und Hillis (18) fanden dagegen bei ihren Untersuchungen über die Reaktionszone fauler Bäume eine deutliche Hemmwirkung von Hydroxymatairesinol und Matairesinol auf F. annosus, während Conidendrin in Übereinstimmung mit Rennerfelt und Nacht (14) nicht hemmte. Das besonders stark hemmende Hydroxymatairesinol kommt jedoch auch im Kern gesunder Fichten teilweise in erheblichen Mengen vor. So ist es nicht verwunderlich, daß gesunde Reifholzproben in unseren Versuchen stark fungistatisch wirkten. Shain (17) hingegen beobachtete nur eine schwache Pilzhemmung des Fichtenreifholzes, untersuchte dabei aber Preßsäfte bzw. wässerige Holzextrakte. Die divergierenden Beobachtungen über die Hemmwirkung des Fichtenreifholzes dürften deshalb teils auf die Eigenart der verwendeten Holzproben, teils auf die unterschiedliche Methodik zurückzuführen sein.

Das Reifholz der Bäume aus dem nur schwach kernfaulen Bestand München-Nord zeigte im Biotest eine stärkere Hemmwirkung gegen F. annosus als vergleichbares Reifholz aus dem Bestand Freising, der extrem rotfäulegefährdet ist. Wir konnten diese Abweichung auf die verschiedenartigen Lignangehalte zurückführen. Dabei waren die Lignankonzentrationen im Reifholz von München-Nord mehr als doppelt so hoch wie in Freising, sie übertrafen auch die Äthanolextraktgehalte des von Shain (17) analysierten Reifholzes erheblich. Unsere Ergebnisse lassen deshalb vermuten, daß die Unterschiede im Lignangehalt des Reifholzes neben anderen Faktoren für die Differenzen im Kernfäulebefallsgrad zwischen den beiden Probebeständen bestimmend sein könnten.

Leider erlaubt unsere Versuchsanstellung nicht zu entscheiden, ob diese Unterschiede auf standörtlichen oder genetischen Faktoren beruhen. Der Versuch scheiterte, die höheren Lignanmengen im Kern (Splint) der Fichten von München-Nord mit den Nährelementkonzentrationen in den Holz-

proben in Beziehung zu bringen; denn auf beiden Standorten enthielten Reif- und Splinthölzer vergleichbarer Herkunft annähernd gleich viel Stickstoff, Phosphor, Kalium, Calcium und Magnesium. Lediglich in den Mangangehalten zeigten sich Standortsdifferenzen, weil die Freisinger Fichten, die auf einem bis zur Bodenoberfläche carbonatreichen Substrat stockten, in ihrem Holz nur Mn-Spuren aufwiesen. Die Ergebnisse einer Faktorenanalyse bestätigten jedoch die Vermutung, daß für unser Material kein kausaler Zusammenhang zwischen der Lignankonzentration (Pilzhemmwirkung) des Fichtenholzes und seinem Gehalt an Nährionen vorliegt.

Wir werden nunmehr in Düngungsexperimenten prüfen, wie weit die Menge und die Zusammensetzung der Lignanfraktion im Holz durch Veränderungen des Ernährungszustands variiert werden können.

Splintholzproben hemmten in unseren Versuchen das Wachstum von F. annosus immer schwächer als Reifhölzer. Sie enthielten weniger Lignane, dafür aber mehr pilzfördernde Stoffe (u.a. Kohlenhydrate und Aminosäuren), welche die fungistatischen Effekte der Lignane weitgehend kompensieren können. Andererseits tragen vermutlich auch petrolätherlösliche Stoffe (z.B. Harzsäuren und Monoterpene) zur Pilzhemmwirkung des Splints bei. Von der stofflichen Zusammensetzung her ist der Splint ein relativ günstiges Substrat für F. annosus. Er ist jedoch im Unterschied zum Kern in der Lage, nach einer Infektion eine besonders lignanreiche Reaktionszone zu bilden, die stark fungistatisch wirkt (7, 17).

Ihr von uns als "grüne Zone" bezeichneter Abschnitt stimmt mit der von Shain (17) gefundenen "Reaktionszone" hinsichtlich Wasser-, K-, Ca-, Mg- und Phenolgehalt weitgehend überein. Allerdings beobachteten wir in keinem Fall das von Shain und Hillis (18) beschriebene ausgeprägte Überwiegen des Hydroxymatairesinols im Vergleich zu den anderen Lignanen. Vielmehr trugen bei unseren Proben aus der grünen Zone alle Lignane mehr oder minder gleichmäßig zur Anreicherung dieser Fraktion bei.

Die zwischen dem intakten Splint und der grünen Zone eingeschaltete "weiße Zone" glich dieser im Phenolgehalt und in der Pilzhemmwirkung, enthielt jedoch deutlich weniger Wasser. Um zu prüfen, ob dieser weiße Abschnitt mit der normalen Übergangszone zwischen dem Splint und Kern gesunder Fichten (16) übereinstimmt, ermittelten wir zum Vergleich die N-, K-, Ca-, Mg-, Petrolätherextrakt- und Methanolextraktgehalte über den Stammquerschnitt eines nicht infizierten Baumes. Dabei zeigte sich, daß die weiße Zone kernfauler Fichten in allen diesen Merkmalen erheblich von der Übergangszone gesunder Probebäume abwich. Somit dürfte es sich um eine spezifische, unter Pilzeinfluss entstandene Bildung handeln.

Die Zahl der verfügbaren Holzproben reichte leider nicht aus, um mögliche Unterschiede im Lignangehalt und im fungistatischen Effekt der weißen und grünen Reaktionszonenabschnitte zwischen den beiden Beständen München-Nord und Freising abzusichern. Wir hoffen nunmehr, die Frage nach dem Einfluß des Ernährungszustandes auf die Ausbildung und die Pilzhemmwirkung von Reaktionszonen durch kombinierte Düngungs- und Infektionsexperimente klären zu können.

Die Untersuchungen wurden durch die Deutsche Forschungsgemeinschaft großzügig gefördert, der wir für ihre Unterstützung aufrichtig danken.

SUMMARY

Healthy and infected Norway spruce trees (Picea abies Karst.) from stands exhibiting greater and lesser degrees of heart rot attack (Freising and München-Nord respectively) were investigated. The stem wood of sample trees was separeted into the following zones:

a) from healthy trees: sapwood and heartwood

b) from heavily decayed trees: sound sapwood, decayed wood and the green and white sections of the reaction zone

c) from less decayed trees: sapwood, healthy and decayed heartwood.

1. Freshly ground spruce wood effected an inhibition of F. annosus in Bioassays. The heartwoods from München-Nord inhibited the growth of the fungal mycelium much more (43 %) than those from Freising (16 %). The white and green sections of the reaction zones inhibited the mycelium growth of F. annosus significantly (each 27 %). The fungistatic effect of sapwood was scarcely noticeable.

2. Longitudinal and trensverse sections of the reaction zone often showed under the microscope a striking accumulation of crystalline deposits, probably of calcium carbonate. In the ray parenchyma cells and in tracheids amorphous inclusions were to be seen, which are thought to be composed of lignans.

3. The density of the samples of sapwood and heartwood was about 0.50g/cm^3. The green and white sections of the reaction zone showed somewhat higher values (up to 0.60g/cm^3). The moisture content of sapwood from both stands varied between 100 and 140 %, that of heartwood between 20 and 40 % of dry weight. The reaction zones contained in the white parts 30-40 %, in the green parts, however 60-70 % water.

4. In corresponding zones of wood from both stands no significant differences in the concentrations of N, P, K, Ca and Mg could be oberserved. In both stands there was a marked increase of K, Ca and Mg in the reaction zones. Heavily decayed trees showed higher concentrations of nitrogen throughout the cross section of the stem.

5. The amount of light petroleum soluble extractives of sapwood and heartwood from both stands comprises 0.5-1.6 % of dry weight. The heavily decayed trees from Freising, already having a rot extending as far as the bark, could be distinguished by their far higher content of light petroleum soluble components (up to 4.8 % of dry weight).

6. Sound spruce trees from both examined stands differed in the content of methanol soluble extractives of their heartwoods. The amount of methanol extracts in the heartwoods from München-Nord was 3-6 % of dry weight, as compared with 1.5-3 % for trees from Freising. In the reaction zone up to 8 % of dry weight of such extractives could be found, in the sapwood in contrast the methanol extract lay between 1.5 and 3.8 %.

Whereas the methanol soluble extractives of heartwood and green and white zones are composed of lignans to an extent of 60-70 %, the same fraction of sapwood has a lower lignan content (36 % in the case of München-Nord, 20 % from Freising). Sapwood samples from Freising had a higher content of carbohydrates and amino acids tha those from München-Nord.

7. By statistical evaluation of our results we could demonstrate a highly significant correlation between the inhibitory effect of wood samples in bioassay and their lignan content.

8. The question of whether the stronger inhibition shown in bioassays of the heartwoods from München-Nord could be responsible for the higher resistance of this stand to F. annosus in vivo was discussed. Also discussed were possible influences of nutrition on wood properties which might be important for the spreading of F. annosus in infected trees. Experiments in order to test this hypothesis are already in progress in our institute.

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INFLUENCE OF TEMPERATURE, pH AND NUTRIENTS ON THE GROWTH OF FOMES ANNOSUS ISOLATES

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INTRODUCTION

Fomes annosus (Fr.) Cooke was discovered in pine forests in Portugal near the end of 1968 and consequently interest in this root rot began only during the three last years. Until 1972 the disease was limited to the Pinus pinaster Aiton stands in the north. The pathogen is progressing to new areas and indeed some other stands of pines especially in Padrela Mountain are seriously infected.

In order to obtain more information about F. annosus, we carried out physiological studies with our strains, particularly in reference to the optimum and lethal temperatures, hydrogen ion concentrations and some nutritional requirements.

MATERIAL AND METHODS

Studies have been done using isolates obtained from different localities of P. pinaster stands. Culture 539 was isolated from trees growing under alkaline conditions; 522 and 522a were isolated respectively from a root and a carpophore on a pine growing under acid conditions.

The technique employed in the physiological studies of these strains or forms (2) was that used in similar experiments (1). Isolates were cultured in medicine bottles of 180 ml capacity, containing malt liquid medium. Bottles containing 10 ml of the media were sterilized by autoclaving for 20 min at 15 atm. pressure. Inoculation was done later using 4 mm agar disks from colonies of Fomes growing on plain water agar plates. Resulting cultures were incubated for 20 days at constant temperatures. The mycelial development of the strains were determined by averaging mycelial dry weights in the experiments for each isolate.

Studies of the optimum temperature were repeated on malt agar.

RESULTS

Temperature

We carried out some trials to establish not only the optimum, but also the inhibitory and lethal temperatures for the development of these isolates. Thus, we have considered values from 19 to 31 C for the optimum temperature; from 3 to 13 C and from 33 to 41 C for the lethal temperatures.

Table 1.--Mycelial dry weights (mg) of isolates 522, 522a and 539 at different temperatures

Isolates	Temperatures (C)						
	19	21	23	25	27	29	31
522	61	61	68	78	69	52	43
522a	60	61	68	78	70	53	47
539	65	90	92	95	102	53	97

The maximum dry weights of mycelium were obtained at 25 C for isolates 522 and 522a and 27 C for isolates 539 (Table 1). These temperature values should be considered the optimum for growth for these isolates and are in agreement with the maximum mycelial growth obtained in similar experiments (3,4).

In reference to lethal temperature values we have tested the isolates at temperatures lower than 13 C and higher than 31 C. We are especially interested in temperatures higher than 31 C because field temperatures are always higher than 30 C in summer when fellings are carried out. According to the results of our experiments we have considered 3 C as inhibitory among low temperatures, and among the high temperatures the value of 33 C. Negative temperatures and all the values above 35 C must be considered lethal to the isolates tested.

Hydrogen ion concentration

Concerning the growth of F. annosus cultures at various hydrogen ion concentrations, we have obtained values in agreement with those of other authors (2). Thus, whereas 522 and 522a have a maximum mycelial weight at pH 4.5, isolate 539 has attained the maximum at pH 7.5 (Table 2). However, 539 shows good mycelial growth at pH 8, too. Mycelial growth at pH 3-3.5 and 5-6 were not significant.

Table 2.--Mycelial dry weights (mg) of isolates in media with various pH's

Isolates	pH values						
	3.5	4	4.5	6.5	7	7.5	8
522	54.5	60	104	98	95	77	62
522a	80	89	100	94	90	80	71
539	75	90	96	117	127	134	130

Nutritional requirements

Emphasizing the importance of the study of substances utilized by F. annosus, some experiments have been done in order to know for certain the nutritional behaviour of our isolates using a standard basal medium (1).

As sources of carbon we supplied the monosaccharides; xylose and glucose, and the disaccharides; maltose and sucrose. The amount of growth presented by isolates 522, 522a and 539 was much better on glucose than on other carbon sources employed (Table 3). It seems clear that Fomes isolates need the presence of carbohydrates for their development, however, all of them prefer to utilize glucose as the carbon source.

Table 3.--Mycelial dry weights (mg) of isolates 522, 522a and 539 on four different carbohydrates sources

Isolates	Carbohydrates				Without
	xylose	glucose	maltose	sucrose	carbohydrates
522	84	132	114.5	113	2.0
522a	46	84.2	50.3	84	5.9
539	137	196.2	180	163	8.2

For a nitrogen source, Fomes, as do other root rot pathogens, had the best growth on peptone. None of the isolates was able to grow on nitrate as the sole source of nitrogen.

Nutritional requirements for calcium, manganese and iron were tested. The nutrient medium was the same employed in the preceding nutritional experiments, except $CaCl_2 \cdot H_2O$ was substituted for $CaSO_4$ in the concentrations of 10, 30 and 100 mg/l (5 and 4). The amount of mycelium of isolate 539 increased with increasing concentrations of Ca up to the maximum amount of Ca we used (Table 4). Mean mycelial dry weights obtained with isolates 522 and 522a were largest at the lowest concentration of Ca and decreased with increasing amounts of Ca. The growth of these cultures in medium without calcium has not presented significant values for dry weights, therefore F. annosus requires the presence of this substance to have satisfactory mycelial development.

Table 4.--Mycelial dry weights (mg) of isolates 522, 522a and 539 on Ca

Isolates	Ca (mg/L)			Without
	10	30	100	Ca
522	181	161	135	96
522a	132	128	109	92
539	62	93	157.5	10

The growth of these cultures in the presence of manganese and iron resulted in no differences when compared with the amount of mycelium obtained on basal medium alone, although these elements are indispensable to the growth of Fomes isolates.

In order to have more information about behaviour of Fomes, some experiments were made employing growth factors such as; Thiamin, Biotin and Piridoxin, tested under three different concentrations: 0.01, 0.001 and 0.0001 ug. According to the results obtained, we must consider that isolates 522a and 539 utilize Thiamin and 522 uses Piridoxin. The results are in general agreement with others (3).

Comparing figures obtained in laboratory trials done in reference to temperature, hydrogen ion concentration, and nutritional requirements, it is possible to find remarkable differences, especially concerning temperatures, pH, some nutritional requirements and growth factors.

SUMMARY

Mean values for the effect of temperature on mycelial weight are similar to those of other authors. As lethal temperatures we must consider all values lower than 3 C and higher than 35 C.

The possibility of different forms of F. annosus developing according to the pH of the soils where the trees are growing, was investigated. The forms tested, 522, 522a and 539, isolated from pines growing on alkaline and acid conditions had the optimum growth at pH 4.5 and 7.5 respectively.

Experiments concerning some of the nutritional requirements, particularly in connection with carbohydrates, nitrogen, calcium, manganese, iron and vitamin sources were done.

The results of these experiments indicate that it is possible to establish differences between our isolates.

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DISCUSSION OF

PHYSIOLOGY OF *FOMES ANNOSUS* INFECTION

Louis Shain, Chairman

INTRODUCTION

Disease physiology is a broad subject covering the physiologies of the pathogen, the host, and the disease process itself. This session, therefore, has been subdivided to reflect these differences in the study of physiology of *F. annosus* infection. The subject of fungal physiology can be further subdivided into the areas of: 1) fungal nutrition with contributed papers by Azevedo and by Negrutsky (the latter was not present), 2) fungal metabolites with the paper by Fedorov and Staichenko (not present), and 3) fungal ecotypes with the paper by Courtois. The subject of host physiology is considered in the paper by Vasiliauskas (not present). In addition, Alcubilla will review some previously published work on preformed inhibitors of *F. annosus* in spruce bark and the influence of host nutrition on the production of these inhibitors. The papers by Alcubilla et al. and Johansson and Theander consider the physiology of the disease process or more specifically, the dynamic host response to infection. In addition to the above, I shall be calling on some people from the floor to contribute to our discussion on related physiological topics.

The basis, or justification, for physiological research is to answer questions that were raised in the session of yesterday afternoon (resistance and relative susceptibility). When we understand the physiology of resistance and susceptibility, we will be in a better position to effectively control our disease problems.

DISCUSSION

Azevedo: Presented paper entitled "Influence of temperature, pH, and nutrients upon the growth of Fomes annosus."

Shain: I am interested in your report that one of your F. annosus isolates, obtained from a tree growing on an alkaline site, grew well at a pH higher than pH 7.0. Several other reports indicate that F. annosus grows poorly at these elevated pH values. Is this the initial or final pH of the medium that you are reporting?

Azevedo: It is the initial pH of the medium.

Shain: Does the pH of this medium decrease during the growth of this isolate?

Azevedo: Yes.

Koenigs: When I was studying the effect of borax on stump treatments, I found that one of its effects was to increase the pH of pine stumps to pH 7.6. It occurred to me that the mechanism of action of borax may simply be a pH effect. I tested this by germinating basidiospores buffered at different pH values. I found on occasion that up to 22% of the spores germinated at pH 8.2. The pH before and after these tests was about the same.

Dimitri: What was the pH at maximum germination?

Koenigs: In that study I was interested in the upper limit rather than optimal pH. Another point I would like to make is that failure to get germination at high pH values may be due to precipitation or chelation of trace elements rather than a direct pH effect.

Shain: Moving on to the topic of fungal metabolites a paper was to be presented by Fedorov on oxidizing enzymes. Since he is not here, perhaps Dr. Cowling could tell us about some of his work on cellulase production by isolates of F. annosus.

Cowling: We have measured variation in amounts of cellulase produced. There is a spectrum. Some are more vigorous than others in producing cellulase, but we never have attempted to associate this with capacity to cause decay or capacity to cause disease.

Shain: Fomannosin, the toxin produced in culture by F. annosus, was discussed extensively at our last meeting. Apparently no additional work has been done in this area since then and the consensus is that none is planned for the future. I would suggest that this is an interesting area that warrants additional research. Another interesting fungal metabolite is hydrogen peroxide. Dr. Koenigs, who has shown an involvement of H_2O_2 in the degradation of cell walls, has agreed to tell us about his work in this area.

Koenigs: I am not actively pursuing the role of H_2O_2 in the metabolism or pathogenesis of F. annosus, but that is how my interest in this area originated. I thought that H_2O_2 would be generated from

oxalic acid which I knew the fungus did produce. The H_2O_2 could then logically activate peroxidases and phenols and, as such, be involved in a pathogenic mechanism. But as my work is turning out, I've been exploring the role of H_2O_2, that is generated by fungi, and iron, that is present in trace amounts in the host, on the degradation of cellulose and lignin. I have found that brown rot fungi generally produce about 12 times more H_2O_2 than white rot fungi. Fomes annosus is a very interesting exception in that it produces nearly as much H_2O_2 as most brown rot fungi. This gives logic to the possible association of H_2O_2 to pathogenesis. When I first started this work about three years ago, I irrigated some pine discs with a suspension of F. annosus conidia. The conidia germinated and grew very well over the cross section of these pine discs but the colonies stopped abruptly at the last annual ring of xylem. But when I flooded the surface of these pine discs with a 1% solution of H_2O_2, the fungus grew over the inner phloem also. I might add that the amount of H_2O_2 that I added was about 10 mg. Some calibration experiments that I've conducted with brown rot fungi indicate that it would take about a month for the fungus to produce this amount of H_2O_2 in wood. This is about all I've done with H_2O_2 and its possible role in pathogenesis. Because the methods needed to demonstrate the participation of H_2O_2 in a living system are complex, I decided to work on wood decay initially.

Peroxide (ca. 6 ppm) with trace amounts of iron (ca. 1.5 ppm) can essentially duplicate the effects of brown rot on wood. This includes rapid depolymerization, increase in alkali solubility, attack on hemicelluloses and oxidation of lignin (particularly hardwood lignin). I have determined that isolates of Lenzites trabea with low decay capacity produce relatively large amounts of phenols. These compounds are strong chelators of iron and perhaps could be considered siderochromes. Demonstration of these strong iron chelators in wood during decay would further implicate the iron-H_2O_2 mechanism in the decay process.

Shain: What about the role of catalase in this system?

Koenigs: If catalase were produced as indicated in the literature, it could destroy the H_2O_2. In fact catalase and peroxidase are probably present in host tissue so that some interaction could be expected.

Johansson: There are peroxidases in sapwood. I believe this could be the basis for defense mechanisms. The logic that is missing from the literature is why peroxidase and phenols are so highly correlated with resistance. Peroxidase is very susceptible to phenols produced in the reaction zone. Perhaps peroxidase is broken down and the H_2O_2 produced by the fungus reacts alone.

Shain: Moving on to the topic of fungal ecotypes, Dr. Courtois will present his paper entitled "About the occurrence of ecotypes of the species Fomes annosus."

Rishbeth: Have you detected any pH differences in spruce tissues growing on the two types of soils (lime deficient and calcareous)?

Courtois: I have not yet studied this question.

Dimitri: Is it possible that there are more than two ecotypes of F. annosus?

Courtois: I have only investigated 15 strains. If we examine additional qualities of strains with other origins, it is possible that more ecotypes will be detected.

Dimitri: Is it enough to examine F. annosus strains under laboratory conditions?

Courtois: No, but after differences are found in the laboratory the next logical step is to test these strains on the host in nature.

Koenigs: I think Dr. Dimitri has a good point. The high decay capacity isolates of L. trabea that I am working with lower the pH of wood whereas the low decay capacity isolates raise the pH of wood above the uninoculated control. In culture, however, both types of isolates lower the pH of the media. False inferences, therefore, can be drawn from extrapolating results from an artificial to a natural medium.

Driver: I would like to know the techniques you used to isolate these strains. Did you isolate them from a single tree or many trees?

Courtois: They were isolated from different trees at different times--one strain coming from one tree. A sample of infected wood was cultured on malt agar. From the mycelium, we took conidia to continue the cultures.

Driver: Were these mass spore isolates or single spore isolates?

Courtois: Because conidia are multinucleate, strains were cultured by mass spore transfers. Experiments were repeated after six months to see if the examined characteristics remained constant.

Driver: Were your results always constant?

Courtois: Not in every manner, but in the manner that I showed you they were quite constant.

Wallis: What is the possibility that the fungus can manipulate its properties in relation to its environment. In other words, if you put the strains from the calcareous site in an acid environment, would it not readjust itself?

Courtois: If the environment is changed for a long period of time, more than 50 years, the strain can readjust or adapt to new conditions.

Wallis: If your strains are able to adapt to a property, I question the use of this property in differentiation of strains.

Courtois: These are only ecotypes or phenotypes and genetic studies are needed.

Cowling: Some time ago we thought about ecotypes of F. annosus and we began to study the simple relationship of temperature-growth response. We found variation, but it was surprising how little variation we found among isolates with as diverse origins as Norway and the southern U. S. We then measured the temperature-growth response of single spores taken from the same sporophore. We found about the same variation within one sporophore as we found among isolates from the whole world. So we came to the conclusion that it is futile to study ecotypes. If we could find so much variation within one sporophore, then what does it mean to compare isolates from Europe and America, etc.

Courtois: But you were observing only one characteristic. It is important to consider broader aspects.

Cowling: Yes, we thought about that too. This fungus has many, many nuclei. So that what we mean by an isolate is a population of nuclei. If these nuclei get separated from each other during spore production, it seems to us that the likelihood is low that a given fungus thallus will maintain a consistent difference. Hence we chose not to continue with this line of investigation.

Courtois: If my results were negative, perhaps I too would have reached the same conclusion.

Cowling: I suggest that you produce sporophores from your strains. If cultures resulting from basidiospores continue to show the same pH relationships, then you have something that is genetically controlled. I hope someone will take up the genetics of F. annosus now.

Koenigs: Supports Cowling's comment with a description of an experiment in which he used single spores from the same isolate in a decay test and obtained wide variation in decay rates.

Rishbeth: I certainly accept what Dr. Cowling has said about the need for further work on genetics but I support Dr. Courtois in his work on pH in particular. I think that in this respect F. annosus probably shows more interesting differences in the field than in any other respect. Early work by Dr. Gibbs, I think, showed quite clearly that there were ecotypes in our area and that they behaved quite differently in culture and in root systems. I think that if this particular criteria had been chosen in other studies, differences would have shown up. My own view, however, is that these ecotypes are rather unstable in nature.

Skelly: Questions Azevedo about the consistency of differences in pH optima between isolates obtained from trees on acid and alkaline soils. Azevedo replies that differences are consistent.

Dimitri: Relates an experience with a single spore isolate from which mutations occurred after vegetative propagation. Such changes could make genetic investigations even more difficult.

Shain: We will now go on to host products and their effects on F. annosus. Dr. Alcubilla will tell us about some previous work on

preformed host products in spruce bark, which adversely effect the fungus (Alcubilla, M., et al. 1971. European J. For. Path. 1:100-114), as well as review the paper presented by her and co-workers entitled "Untersuchungen uber die Pilzhemmwirkung des Fichtenholzes (Picea abies Karst.)."

Yde-Andersen: Have you studied the breakdown of wood from sound trees growing in your different plots to see if there is a significant difference in decay rate?

Alcubilla: We have observed differences in heartwood. In addition to passive resistance there is also active resistance.

Yde-Andersen: You have found materials in the wood that are antagonistic to the fungus, but do they work when you try to infect wood blocks?

Alcubilla: We have inoculation experiments to study this.

Shain: Perhaps I can answer part of your question. I found that the reaction zone was significantly more resistant to decay by F. annosus than either sound sapwood or heartwood.

Yde-Andersen: Have you tried to make isolations from the reaction zone to see if there are any microorganisms in it? In my experience bacteria are often found in this zone.

Aufsess: This is our experience also.

Yde-Andersen: Do these bacteria have any effect on the growth of F. annosus?

Aufsess: We have not yet studied this.

Shain: What you call "white reaction zone" I have called transition zone. I tend to favor the latter terminology because, unlike the necrotic reaction zone, this light colored zone is metabolically active and it does serve as a transition between sapwood and reaction zone.

Cowling: Yesterday, we saw some pictures of a spruce stem with a hole in the middle. That suggests that Fomes rotted the center but was unable to spread further. Is this a common observation in Germany?

Alcubilla: Yes.

Cowling: Do you think that the concentration of decay in the tree's center is controlled by reaction zones?

Alcubilla: This can be, but we must examine more trees. The reaction zone has a high lignin content.

Cowling: Let me ask Shain to make a distinction between barrier zones and reaction zones.

Shain: The barrier zone is characterized by the fungus invading only the tissues that were present at the time of wounding. Xylem produced after wounding is not attacked. In P. radiata attacked by the Sirex-Amylostereum complex, we found that injured cambium produced a metabolically active wound parenchyma. Upon necrosis, these cells produced considerable amounts of pinosylvin. Such activity apparently provides a barrier against the fungus moving into newly-formed tissue. A reaction zone occurs when the fungus becomes established in the interior of the tree. As the fungus moves outward, it affects adjacent sapwood. As sapwood parenchyma die, inhibitory compounds are produced.

Cowling: The thing that I would like to point out is that the reaction zone is a dynamic phenomenon going on in already existing tissues. Barrier zones are fixed barriers laid down by the cambium at the time of wounding. It is possible that heartrot in spruce can be reduced by wounding trees to induce barriers through which the fungus cannot pass. We know this works in angiosperms and Shain has demonstrated that this phenomenon exists in pine also. Fixed barriers also seem to have been observed in spruce. So, doesn't this raise the possibility that we could learn to induce barriers in spruce and, in so doing, limit decay to within the barrier zone. Shigo has developed a non-destructive technique (pulsed electric current) to identify trees with and without heartrot. We should think about these analogies with what goes on in angiosperms. Perhaps there are some interesting possibilities.

Shain: A fresh wound could nullify the effects of an earlier barrier zone.

Cowling: Perhaps we can learn to induce barriers from time to time by wounding aseptically.

Dimitri: Because it is difficult to produce wounds aseptically, your suggestion may lead to the greater problem of Stereum infection.

Koenigs: Shigo has done these types of experiments for years and he has not been able to wound a tree without introducing microorganisms into the wound--at least sometimes afterward he isolates organisms other than the ones he introduced. It may be that microorganisms are producing this "heartwood" rather than other agencies. Have you isolated to determine if other microorganisms were there?

Shain: I have described isolations of this kind and it appears that reaction zones can be produced in the absence of microorganisms even though they are frequently present. But, it is difficult to prove a negative. It is possible that some fastidious microorganisms were missed. I am not aware of studies indicating the presence or absence of microorganisms in barrier zones.

Yde-Andersen: Have you tried to inoculate bacteria into sound trees to see if they are able to form this reaction zone?

Shain: My assessment of the reaction zone is that it is a nonspecific response to wounding.

Johansson: We have tried to induce reaction zones several ways, e.g., nails, culture filtrates, insertion of small pieces of reaction zone. These attempts failed due to resin accumulation.

Shain: I think you would have succeeded in inducing reaction zones if your wounds penetrated older wood where resin accumulation does not occur.

Johansson: Presents paper entitled "Effects of extractives from spruce roots on extracellular enzymes of Fomes annosus (Fr.) Cke."

Wallis, Cowling, Dimitri: Rediscuss barrier zones.

Russell: A severe freeze in the winter of 1955 killed back many western hemlocks. These trees have a small core of heartwood which apparently has not progressed any further. At first we thought this was F. annosus infection but it appears to be freeze damage. This could be a resistance phenomenon induced by a form of wounding.

Srago: Has anybody looked to see if a barrier is formed in response to mistletoe infection?

Shain: Tainter has reported the formation of wound periderm in response to mistletoe infection.

I would just like to mention a few areas that I think will assume importance in the next few years with regard to host resistance:

(i) With forest fertilization becoming more widespread, the influence of fertilization on resistance will need greater attention.

(ii) Protective enzymes produced by the host. Two papers, presented earlier this month at the meetings in Minneapolis (2nd Int. Congress of Plant Pathology), described host-produced enzymes which degrade the cell walls of pathogenic fungi. In one of these papers an inhibitor of these enzymes, produced by the pathogen, was reported. So again, we see evidence of the continual action and reaction of host and pathogen.

(iii) Ethylene production could provide a rapid test for distinguishing resistant from susceptible hosts.

In our remaining time, perhaps we can discuss the very basic question: how are trees killed by F. annosus? More specifically, what vital process of the host is sufficiently interfered with to cause mortality (particularly in pine) and how does the pathogen interfere with this vital process?

Rishbeth: It seems to me that many but probably not all of the symptoms could be associated with stopping the water supply.

Cowling: This hypothesis can be tested by measuring the influence of this fungus on transpiration. I would encourage all of us to consider undertaking such experiments to specifically demonstrate what happens after infection in relation to a very important physiological process--uptake of water from the soil.

Skelly: Windthrow of infected trees while they are still green supports the hypothesis that water uptake can continue even perhaps through a decayed root. I think the mechanism of killing is largely related to the degree of infection of the roots.

Shain: But how does this root infection affect the tree?

Skelly: Perhaps it could be related to water potential. This may explain "low" and "high" hazard sites because drought and moisture stress occur more frequently on sandy soils.

Hodges: The decrease in growth rate on low hazard sites in Virginia indicates to me that these trees are infected over a long period and trees decline for a few years before death. Trees on high hazard sites die so quickly (1-2 years) that they usually don't have a chance to reflect loss in growth. I attribute death to the killing of the cambium of almost all the roots with the ultimate disruptions of water and mineral uptake.

Parmeter: I think we have to be careful in assuming there is only one mechanism involved in tree mortality. Our experience with white fir suggests that most of the mortality is associated with bark beetle attack. Fomes annosus seems to trigger susceptibility to bark beetle attack. Add to this the interaction of environment, and we have a complicated picture. I don't think it will be easy to say this tree dies because ---.

Witcher: It seems to me that southern pine are killed in two distinct ways: (i) They fall over and the roots dry up and they eventually die; and (ii) they die when they are firmly anchored. Most dead trees that we examined have quite a bit of resin infiltration which could be the way water uptake is impaired.

Russell: Questioned the group about predisposition of a given tree for attack leading either to mortality or to butt rot without external symptoms. No response was given to the question.

Froelich: I'm sure that many of you observed the trees ability to produce new roots. In cases of extensive root decay, lateral roots can serve as tap roots. These lateral roots, however, are not strong enough to anchor the tree in a hurricane. The ability of the tree to produce new roots, which I think is largely a function of site, could be very important during the infection process.

Rishbeth: This is an extremely important point. Resistance mechanisms associated with secondary cambial activities would include generation of new roots as well as cambium-formed barriers. These responses as well as others (e.g., resin accumulation) would be part of the trees defense arsenal.

Redmond: We found that spruce is particularly good in regenerating its roots after attack by Armillaria. When Sitka spruce is planted on wet peat, most of the root system dies and a new root system is produced just below the surface.

Shain: No one has invoked the toxin theory yet.

Cobb: Frankly, I do not dismiss the toxin effect as one of the causes of death in these trees. I'm not saying it is fomannosin, but I think there is an effect of the fungus in addition to any effect on moisture uptake. Fomes annosus triggers the susceptibility of ponderosa pine to bark beetle attack. Dramatic wounding experiments (wounds into heartwood), on the other hand, have not induced bark beetle attack.

VARIATION IN INFECTION OF LOBLOLLY PINE ROOTS ON HIGH AND LOW HAZARD SITES IN THE SOUTHEASTERN UNITED STATES

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Pine stumps on low hazard sites are as frequently infected by Fomes annosus as stumps on high hazard sites; however, the disease spreads more readily into the residual stand on the high hazard sites. In England, Rishbeth (4) found more frequent root infections as well as a greater capacity for growth on the bark of roots on high hazard alkaline sites than on low hazard acidic sites. In the southeastern United States, high hazard sites are well drained, deep sands and low hazard sites are sands with high water tables or heavy clay. The soil reaction varies less than 0.5 pH units on these sites in the southeast whereas differences of 1-5 pH units occur in England (4). On high hazard sites in the southeast, the stumps decompose rapidly and are nearly disintegrated in 5-7 years, whereas on low hazard sites the stumps remain solid after 5-7 years. This study was undertaken to compare the frequency of root contact and root infection on a low and a high hazard site.

METHODS

The high hazard site was on a well drained, deep sand of the coastal sandhills near Aiken, S. C. The 20-year-old loblolly pine stand (Pinus taeda L.) had been planted on a 1.8 x 1.8 m spacing and was

thinned 4 years prior to sampling. The low hazard site was poorly drained, with acidic fine sandy loam underlain by firm sandy clay, on the coastal plain near Aulander, N. C. The 26-year-old loblolly stand had been planted on a 1.8 x 2.4 m spacing and was thinned 6 years previously as part of another study (5). On both sites approximately half of the stumps had conks of F. annosus. Roots of these infected stumps that were oriented toward the closest residual tree were excavated to determine the frequency of: contact between roots of stump and tree; host response of residual tree roots as indicated by signs or symptoms of infection; infection of the tree roots as indicated by isolation of F. annosus on agar medium from wood and bark chips; and death of the residual trees. If contact between roots was observed and a host response had occurred, the distance from the stump to the root contact was estimated.

RESULTS AND DISCUSSION

Root systems of 348 stumps on the low hazard site and 255 stumps on the high hazard site were examined. A comparison of the data for the two sites indicates that the high hazard site had a higher frequency of occurrence in every category than the low hazard site (Table 1). The factor contributing the most to the difference in disease development was the greater frequency of root contact on the high hazard site. Root contact occurred nearly three times more frequently on the high hazard site than on the low hazard site. Even when root contact occurred, the fungus did not infect the residual tree roots as readily on the low hazard site as on the high hazard site. The ratio of tree roots infected/root contact is 10% on the low hazard site and 43% on the high hazard site. Similarly, infection resulted in less mortality on the low hazard site than on the high hazard site.

Table 1.--Comparison of root contact between infected stumps and residual trees and infection of residual loblolly pines on high and low hazard sites

Site hazard	Stumps (no.)	Root contact (%)	Signs or symptoms[a] (%)	Roots infected[b] (%)	Trees dead (%)
Low	348	28	14	3	0.3
High	255	69	46	30	9.0

[a]On residual tree roots.
[b]Fomes annosus isolated from residual tree root.

Depth to water table has been shown to have a marked effect on the root system which would influence the frequency of root contacts. Monk (3) reported striking differences in the root systems of naturally seeded loblolly pine on a soil with an April water table depth of 15-30 cm versus those on soil with a water table depth of 3 m. The roots from the former were short and stocky while the latter had long roots. White et al. (7) found that slash pine (Pinus elliottii Engelm. var elliottii) with fluctuating shallow water tables had half the root biomass that trees with 46- or 92-cm depth water tables had after 5 years. These differences were due to greater numbers of lateral and tap roots, not of fine roots. The reduced root systems on the wet, low hazard site resulted in fewer opportunities for root contact than would have occurred on the well drained, high hazard site.

Having more roots should result both in more root contacts and more root grafts. Because root grafts first require root contacts, the relative occurrence of root grafts should indicate the frequency of root contacts. Root grafts occurred with seven stumps on the high hazard site and three stumps on the low hazard site. This frequency of root grafting was less than the 7-15% Shultz (6) reported. However, stumps, grafted to live trees remain alive, are less likely to be infected by F. annosus (1, 2), and were thus less likely to be excavated in this study. Shultz' data (6) do suggest the difference in spacing in my study was not sufficient to cause the reduction in root contacts. In slash pine the percentage of grafted, living stumps was 7.6 in plantations spaced 1.8 m within rows. This percentage gradually decreased to 4.0 in plantations with 3.0 m within row spacing (6). On the low and high hazard sites the between row difference of 0.6 m does not seem sufficient to have nearly tripled the frequency of root contact.

The average length of the stump roots from the stump to the point of contact was similar for tree roots having signs or symptoms on both sites (Table 2). This further indicates that spacing differences in this study did not affect the distance to the point of contact. The distribution pattern in the infected and non-infected categories was similar indicating infection does not occur more readily when contact occurs close to either the stump or the tree.

Rishbeth (4) found that F. annosus failed to infect pine roots on acidic sites in England as frequently as it invaded roots on alkaline sites. A similar failure occurred on wet, low hazard sites in the southeastern United States since the fungus was not isolated as frequently from roots in contact with infected stumps on low hazard sites as on high hazard sites. Even when infection occurred, death was less frequent. The slower rate of decay in the wet soil would seem to present a longer opportunity for the fungus to move to the tree root but other factors prevent this, as well as growth on or in the tree roots. The residual tree roots were examined for several feet proximally and distally to the point of contact for evidence of infection; however, the host responses were most evident at the point of contact. Isolations were made only from wood and bark that had signs or symptoms of infection.

Table 2.--Comparison of distance from stump to point of contact with tree roots in various stages of disease development

Site hazard	Disease development			Distance (m)							Average length (m)
	Signs or symptoms	Roots infected[a]	Tree live (-) or dead (+)	0	.3	.6	.9	1.2	1.5-1.8	2.1-2.4	
				Number of stumps							
High	+	-	-	9	8	9	13	7	8	1	0.8
High	+	+	-	4	7	7	18	6	3	5	1.0
High	+	+	+	3	0	4	3	7	4	1	1.0
Low	+	-	-	8	4	5	4	6	8	1	0.8
Low	+	+	-	1	0	1	4	2	1	0	0.9

[a] *Fomes annosus* isolated from residual tree root (+) or not isolated (-).

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DISEASE INCIDENCE AND DISEASE SEVERITY IN LOBLOLLY PINE PLANTED OVER TWO SOIL HAZARD TYPES

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INTRODUCTION

The importance of annosus root and butt rot to the Southern forest industries of the United States is well documented. Due to the rapid increase in the numbers of loblolly pines (Pinus taeda L.) planted and resultant thinning operations, the potential of this fungus incited root rot is expected to increase.

Hodges (5) has elaborated on many modes of infection and spread of F. annosus. He reported an abundance of conflict within the area of edaphic factors as related to this spread.

Certain soil factors have consistently been associated with Fomes annosus damage in pine plantations. The incidence of F. annosus has been shown to be higher by many workers on soils with high sand content and organic matter (4, 10, 11, 14). Froelich et al. (4) have characterized severely damaged plots as having 0.40 to 1.59 percent organic matter, 71 to 90 percent sand, 0 to 10 percent clay, 0 to 10 percent grass cover, and a pH range of 5.4 to 5.9. Morris and Frazier (10) developed a hazard rating system in which high hazard sites have above 70 percent sand in the top 20 to 30 cm and a water table below 45 cm. Towers and Stambaugh (16) have shown that low soil moisture affects the incidence

of the disease. Rishbeth (14) obtained infection of pine stumps with soil taken from soil depths of 50 to 90 cm. and has shown that moist quarry sand may become infested with basidiospores when exposed to the air for 3 months. Kuhlman (7) has shown that conidia could survive up to 10 months in forest soils of the White Store series and that conidia percolation occurs in sandy soils when the 0 horizon has been removed. Molin (11) demonstrated that spores could be washed through a column of sand. Rishbeth (14) and Wallis (17) have demonstrated that higher infection rates occur on alkaline soils than on acid soil. This study was designed to further elucidate the levels of infection on two soil site extremes and to relate the previously studied soil factors to these levels of infection.

MATERIALS AND METHODS

The plantation was established in 1940 over two extremely different soil hazard types. All cultural practices including thinning operations which were conducted in 1957 and 1966 were carried out uniformly over both sites. Morris (9) has reported that the mortality rate, 6-yrs after initial thinning for this plantation, as being 1.3 cords and 2.4 cords for the low and high soil hazard types respectively. The high soil hazard type (HIGH) was initially determined to have texture composed of 73% sand, 23% silt, and 4% clay and the low soil hazard type (LOW) was found to be composed of 43% sand, 47% silt, and 10% clay (1). All isolations reported in the studies below were made onto OPP media (15) and plates were incubated at 24 C for 14-21 days. A 0.02 ha plot was established on each soil type within the plantation. The two plots were located immediately adjacent to stumps or trees exhibiting a conk of F. annosus but the plots did not include this first found conk on either soil hazard type. Each tree was numbered, the d. b. h. recorded, and rated for crown symptom expression (disease severity) as follows: 1-dead; 2-thin, chlorotic crown; 3-thin, green crown; 4-healthy; trees with conks were also counted and recorded. Increment core samples were taken from the stem-collar zone of two roots for each tree. The cores were plated onto the OPP media in the same fashion as reported by Cordell and Stambaugh (2). Based upon obvious discrepancies of disease severity as evidenced by crown symptom and the presence of conks of the fungus, it was decided that the isolation results were not indicative of the amount of F. annosus incidence in the sample plots. Therefore (12-15 weeks later) 45 cm samples of the same two roots on each tree were excavated, excised, and chip isolations were made from specific symptomatic areas along the root section.

Using a controlled sampling procedure a second study was initiated to determine the incidence of F. annosus for a larger portion of the high and low hazard areas of the plantation. From a randomly selected starting point, two roots (45 cm long) were excised from every fifth tree for 5 trees in 2 rows spaced 5 rows apart (10 trees sampled), and chip isolations made.

A third study was conducted to determine the incidence and location of F. annosus in the root systems of 10 trees; five in each soil hazard plot. These trees were located throughout each plot and their entire root systems were excavated out to a root diameter of 0.5 cm or less.

Each root was numbered and measured for length, depth, and direction. All root contacts and grafts were recorded. They were then excised and removed to the laboratory where chip isolations were made every 10 cm from the wood of top and bottom side of each root.

Soil data for each plot was taken at plot center from depths of 10, 20, 30, 60, 90, and 120 cm. The soil texture was determined by the pipette method of analysis (15). Porosity and field capacity were determined by removing duplicate ring core samples from each of the six depths and measuring the amount of water present at pressures of 60 cm, 0.1 bar and 0.33 bar using the moisture extractorceramic plate and difference method. Permanent wilting point was measured at 15 bars. The North Carolina method of extraction was used to determine the amount of Ca, Mg, P and K in the soils (13). The pH was determined using a 1:1 soil to water mixture. Organic matter was determined by wet digestion with sodium dichromate and sulfuric acid (13). Bulk density was also measured for the soils.

RESULTS

The number of trees, their average d. b. h. and disease severity rating are summarized for each plot (Table 1).

Table 1.--Number of trees d.b.h., and disease severity rating of the two 0.02 ha plots

Plot	No. trees	cm D.B.H.	Rating
HIGH	11	21.0	2.6
LOW	12	24.3	3.6
Diff.		3.3	1.0

There were no conks present in the plots. Percent infection with the increment core samples and two root samples were 5.0 and 36.3, respectively, for the HIGH plot. No infection was found in the LOW plot with either method (Table 2). Similar results were obtained from the second study involving the 10 tree random samples, in that 30 and 0 percent infections were found in HIGH and LOW plots, respectively (Table 3). The excavation of the root systems of 5 trees on each plot demonstrated that 4 of 5 on the HIGH and 2 of 5 on the LOW plots were infected. The comparison of all methods of determining the incidence of F. annosus in these 5 trees/plot is summarized in Table 4. These data are based upon 97 roots excavated from the 10 trees, 44 from the HIGH and 53 from the LOW plot. A total of 2625 root chips were taken from depths of 0 to 95

Table 2.--A comparison of the percent recovery of F. annosus from all trees/plot using different techniques

Plot	No. trees	No. conks	Increment borer	2 Roots
HIGH	11	0	5	36.3
LOW	12	0	0	0

Table 3.--The recovery of F. annosus from the two hazard sites

Area	No. trees	No. positive	% Infec.
HIGH	10	3	30
LOW	10	0	0

Table 4.--Increased recovery of F. annosus as demonstrated by different techniques

		Number Trees Positive						
Plot	No. trees	Conks	Increment cores	2 Roots	%	All Roots	%	% increase using all roots
HIGH	5	0	0	2	40	4	80	40
LOW	5	0	0	0	0	2	40	40

cm. However, only those chips taken from roots located at the soil sample depths are recorded in Table 5 along with the various soil parameters measured. The percent infection of all roots was 5.8 in the HIGH plot (89 of 1552 chips) and 1.1 in the LOW plot (12 of 1073). Of the 44 roots from HIGH, 16 (36%) were positive, and from LOW, 4 (7%) of 55 roots were positive. Infected root chips were found in HIGH at depths of 5 to 95 cm, and in LOW at depths of 5 to 45 cm. At depths of 5 to 30 cm, the total number of chips removed were 1335 and 956, with 44 (3.0%) and 7 (0.7%) chips positive for HIGH and LOW respectively. From 35 to 95 cm deep, the total number of chips removed were 217 and 117, with 45 (20.7%) and 5 (4.3%) chips positive for HIGH and LOW, respectively. The average percents of soil parameters at depths of 10 to 30 cm are listed in Table 6.

Table 5.--Soil parameters by depth of removal and the infection patterns of roots found at these levels for HIGH and LOW sites

Depth cm	Percents							Total Number Chips
	Sand	S. Pores	L. Pores	F.C.	Clay	O.M.	Inf.	
HIGH								
10	76	16	23	9	3	1.2	0.4	438
20	76	17	27	10	3	1.1	12.0	100
30	70	18	26	8	4	0.3	12.3	65
60	66	20	17	11	10	0.2	75.0	4
90	68	20	21	12	6	0.2	0.0	5
120	93	27	11	15	3	0.2	0.0	0
LOW								
10	53	30	15	16	11	3.7	0.4	245
20	59	27	15	14	14	3.1	0.3	316
30	65	25	10	11	9	2.2	1.3	76
60	63	25	12	12	9	1.4	0.0	7
90	56	33	10	16	20	0.9	0.0	0
120	66	29	10	13	21	0.9	0.0	0

Table 6.--Average percent soil data at 10-30 cm depths for HIGH and LOW plots

	HIGH	LOW
Sand	74	59
Silt	22	29
Clay	4	11
S. Pores	14	28

Table 6.--(Continued)

	HIGH	LOW
L. Pores	26	14
F.C.	9	14
P.W.P.	2	4
pH	4.6	4.9
O.M.	0.8	3.0

The majority of the roots in the HIGH plot were located in the top 15 cm with few fine branch roots and root grafts. In the LOW plot, the majority of roots were short with many fine branch roots and no root grafts.

DISCUSSION

In the HIGH plot, an average loss in d.b.h. of 3.3 cm was found as compared to the LOW plot. An average increase in disease severity of 1.0 units was found in the HIGH plot over the LOW plot. Morris (10) found the loss in mortality in this plantation to be 0.4 standard cord/acre/yr; measured 9 yrs after the first thinning, with an average loss of $2.40/acre/yr during this same period.

A comparison of the different methods utilized for determining the incidence of infection, points to a possible inaccuracy in our field methods for determining infection when the high disease severity classes were very much in evidence. The increase infection in this study was at least 30% higher than many previous reports and probably much more if a 40% increase is added on to the two root sample percentages. Although the two root method is much more destructive than the increment borer method or counting conks, it is much more reliable for accurate determination of infection. Dimitri (3) has shown a 45% increase in diseased trees with slices as compared to boring cores in butt rot of spruce.

Sand was found to be the most significant soil factor, as was demonstrated by Morris and Frazier (10). The soil parameters generally agreed with those found by Froelich (4) except for pH. The most significant soil parameters were sand, small pores, large pores, field capacity, clay, and organic matter. Sand and large pores were found to be directly related to infection, with small pores, FC, clay and organic matter inversely related to infection.

Rishbeth (14) reported finding spores 90 cm deep in sandy soils, and Kuhlman (7) reported that conidia could remain viable for up to 10

months in clay type soils. Kuhlman (8) also reported that as few as 44 spores/root could cause an infection of nonwounded roots and that with the O horizon absent, conidia percolated to a depth of 15 cm. Although no isolations were made from the soil, infected roots were found down to 95 cm in HIGH and 45 cm in LOW. The highest percent infection in HIGH was 60 to 100% at depths of 60, 65, 70, 85 and 95 cm, while in LOW the highest percent infection was 25% at 45 cm. The presence of infection at these levels points to the possibility of spore percolation being a source of inoculum. This possibility was previously suggested by Rishbeth (14) and Kuhlman (7). The high amounts of sand and large pore space in the HIGH plot tends to increase the possibility of spore percolation, due to the size of the pores and the relatively rapid movement of gravitational water through these types of soil. This trend (Table 5) of increased percent infection with increase in depth in the HIGH was not evident in the LOW plot. Only one root graft was found in the HIGH plot to be involved in transmission of the fungus and this was between a stump and root. None were found in the LOW plot.

The unique nature of the plantation as described previously has provided the opportunity to measure these various influences on the disease incidence and severity of symptom expression in loblolly pines located on two soil hazard types. Previous reports of disease incidence are most probably very conservative and F. annosus may be of considerable more importance than heretofore thought.

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EFFECT OF TEMPERATURE ON GROWTH RATE AND SURVIVAL OF FOMES ANNOSUS IN WESTERN HEMLOCK STUMPS

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INTRODUCTION

Fomes annosus is endemic to the Pacific Northwest region and is reported primarily as a cause of butt-rot of mature western hemlock (Tsuga heterophylla [Raf. Sarg.]) trees. Generally, increase in occurrence of F. annosus is related to increased intensity of forest management, and western hemlock is destined to become intensively managed.

In the southeast U.S.A. and Europe spread of the fungus primarily occurs by air-borne spores which infect freshly cut conifer stump surfaces during thinning operations. The fungus then spreads to the remaining stand through the root system by means of root grafts or close contacts.

Stump infection by F. annosus following thinning of young western hemlock stands has been demonstrated by Driver and Wood (1968) Edmonds, et al. (1969) and Edmonds and Driver (1970). Increased stand infection associated with intensive forest management of western hemlock stands

is thus likely.

Air temperature at the time of felling was revealed as an important factor affecting conifer stump colonization. Little infection occurred when thinnings were conducted while the mean daily temperature was above 21° C (Driver 1963, Driver and Ginns 1967, Ross 1967, Ginns and Driver 1970) or below 0° C (Yde-Anderson 1962). Stump infection was favored when temperatures fell between these two extremes (Stambaugh et al. 1962, Boyce 1963, Driver and Ginns 1964, Sinclair 1964, Ross 1973). Driver and Ginns (1969) have indicated that the mechanism of the temperature effect is not solely due to thermal inactivation of the fungus, but an interaction between ambient temperature and resulting stump temperature, spore production, and the presence of competing fungi.

The research reported here deals with the effect of temperature on growth rate of mycelium of F. annosus in western hemlock stumps and germination of conidia on the stump surface. Linear growth rates of isolates of F. annosus and several competitors were also determined on artificial media at various temperatures. Stump surface and internal temperatures were investigated in both western hemlock and Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco.) stumps at several locations in western Washington throughout the year, and determined in simulated stumps at the laboratory.

MATERIALS AND METHODS

Growth Rate Determination in Stumps

Stumps which had been exposed in February, May, June and September at Clallam Bay (Figure 1) were sampled 20, 18, 17-1/2, and 15-1/2 weeks after cutting, respectively, and transported to the laboratory. These stumps were selected randomly in such a way as to effectively sample stumps of all diameters. Twenty-five stumps were sampled from each of three plots in February, May, and September. Only ten stumps in total were sampled in June.

The stumps were cut into 2.5 to 5.0 cm sections on a band saw, numbered serially, wrapped in newspaper, and placed in ventilated 20 gallon cans to incubate. The sections were examined for the Oedocephalum stage of F. annosus after 14 days incubation. The distance from the top of the stump to the last occurrence of F. annosus in the serial sections was measured to obtain an approximation of the depth of penetration at the time of sampling.

Inoculation of Stem Sections with Conidia of F. annosus

The goal of this approach was to determine the stump surface temperature at which conidia of F. annosus are thermally inactivated, and the number of hours at this temperature which are required to inactivate them.

Stem sections from small hemlock trees approximately 2.5 cm d.b.h. were cut into 1.3 cm sections on a band saw, placed in a polyethylene bag and sterilized using propylene oxide. To accomplish this, dental plugs saturated with propylene oxide were sealed in the bag with the sections for 24 hours and the gas was then allowed to escape. The sterilized sections were then placed on wire racks in sterile plastic containers with 200 ml of sterile distilled water in the base. The containers were then placed in incubators at the test temperatures for at least six hours before they were atomized with conidia of F. annosus.

Conidia of F. annosus were obtained by placing five ml of sterile distilled water in a 6 to 7-day-old tube culture of F. annosus on 2% malt agar. The water was gently swilled in the tube so that very little mycelium would be broken and then filtered through a sterilized polypropylene filter (Gelman - pores size 10 μ) under vacuum. This ensured that no large pieces of hyphae would be included in the filtrate, but conidia (approximately 5 μ in diameter) would be allowed to pass with ease. The filtrate was transferred to a sterile atomizer and the sections were atomized in the incubators stored at the test temperatures. The sections were incubated at the test temperatures for 1, 7, and 12 hours, except for the 0°C test, which was incubated 7 days, and then transferred to the 25° C incubator to allow for activation of growth.

Linear Growth Rate Studies

Linear growth rates were determined from 0-30° C at 5° C intervals for seven F. annosus, one Graphium sp., one Leptographium sp., and one Trichoderma sp. tissue isolates from western hemlock. The latter three fungi were commonly isolated from hemlock stumps.

Growth rates were determined in 17.5 x 2.0 cm dam culture tubes. Growth media was that described by Cowling and Kelman (1964). Eleven ml of the media were placed in each tube by means of an automatic pipette. The tubes were inoculated with agar plugs taken from the edge of an actively growing 4 to 7-day-old culture on 2% malt agar plates, by means of an inoculating punch. The tubes were incubated at 25° C for 3 days to overcome the lag phase of growth, before being transferred to the test temperature. A growth tube reader similar to that described by Clark (1962) was constructed and measurements were made every 24 hours at 20 and 25° C and every 48 hours at 0, 5, 10, 15 and 30° C. The range 0-30° C was studied because Cowling and Kelman (1964) had found that F. annosus grew little at 4 and 30°C.

Stump Temperature Determinations

(a) Field studies

Two sites were selected for continuous measurement of stump surface and internal temperatures. These sites were the Charles Lathrop Pack Demonstration Forest, University of Washington (Pack Forest), which is situated approximately 70 miles south of Seattle,

Washington, and the Cedar River Watershed, situated approximately 40 miles southeast of Seattle (Figure 1). Due to the lack of facilities, temperatures were measured on one day only at Clallam Bay.

Pack Forest. A western hemlock and a Douglas-fir tree were felled in a stand opening, leaving stumps 14.7 and 16.5 cm in diameter, respectively, and 30.5 cm high. The stand opening was approximately 30.5 meters in diameter and was surrounded by trees approximately 19.8 meters high. The floor of the opening was approximately level. The Douglas-fir stump was situated on the northern edge of the opening and the hemlock stump was situated approximately 6.1 meters from the Douglas-fir stump in the northwest quarter. The stumps were in full sunlight for most of the day.

Thermistor probes (Fenwal GB22P32, R_o 2,000 ohms $\pm$ 5% at 25°C, -3.9% per degree C, 0.25 cm diameter, and 1.3 cm length) were used for determining stump temperatures. The probes were calibrated in an oven from 0-50° C, using a platinum resistance thermometer as a standard. To reduce radiation errors in surface temperature estimates, the probes were surrounded by toothpicks and then covered with aluminized mylar. The toothpicks permitted a free flow of air around the probes.

The surface probes were attached with a small wire staple so that only the probe tip touched. Small holes were drilled into the center of the stumps 2.5, 7.6, and 15.2 cm below the surface, and probes were pushed into the holes until good contact was made.

Ambient temperatures were recorded in the canopy, using shielded probes. Maximum and minimum ambient temperatures recorded at La Grande, half a mile distant, were used for comparison.

The system involved wheatstone bridges and millivolt outputs to a Leeds and Northrup Speedomax G strip chart recorder with a range of 0-10 millivolts, which was equivalent to 0-50° C. A common calibration curve of millivolts versus temperature degrees C was complied for all eight thermistors. The temperature value for each 0.1 millivolt was transferred to a scale and temperatures were read from the strip charts at hourly intervals, although values were printed out every 20 minutes. Maximum and minimum temperatures were also recorded. A separate scale for ambient temperatures was used as a different calibration curve was involved. Maximum error involved in the system was calculated to be $\pm$ 0.5° C.

Temperature measurements began in August 1967, and continued until January 1968.

Cedar River. The same type thermistor probes as those used at Pack Forest were used and calibrated in the same manner, but from 0-100° F (0-37.8° C). Surface temperature probes were treated in the same manner. Two Douglas-fir trees were felled -- one in the stand, the other in the open -- leaving 15.2 cm high stumps, and probes were placed on the surface and 7.6 and 15.2 cm below the surface. The 21.6 cm diameter stump in the open was situated on the northeast edge of a 20.1 meters square approximately level clearcut, and the 20.3 cm diameter stump in the stand was under the canopy of a Douglas-fir planation approximately 21.3 meters high.

The system involved wheatstone bridges and millivolt outputs to a punch tape recorder. The output was approximately linear, 0-100 millivolt outputs to a punch tape recorder. The output was approximately linear, 0-100 millivolts being equivalent to 0-100°F. Millivolt values were recorded at two-hour intervals and temperature values were computed from the punch tape, Fahrenheit values being converted to Celsius.

Temperature measurements began in August 1974 and were continued through January 1968.

Clallam Bay. Surface and internal stump temperatures were measured on August 23, 1967. Thermistor probes of the same type used at Pack Forest and Cedar River were calibrated from 0-50° C in the calibrating oven, and placed on the stump surfaces and in holes drilled 7.6 and 15.2 cm below the surface of the three stumps 2.5, 4.8, and 15.2 cm in diameter, within a radius of 6.1 meters. Surface probes were treated similarly to those at the continuously recording sites.

Thermistor resistance values were determined with a Simpson 269 Series 2 Ultra High Sensitivity-Volt-Ohm-Microammeter at hourly intervals during the day, and temperatures were read from the calibration curves. Sunny conditions prevailed during the day.

(b) Laboratory studies. Stump temperatures studies in simulated stumps.

This approach was attempted to determine the maximum stump temperatures that could be attained when hemlock stumps remained in the sun for long periods on a hot day.

Young hemlock trees of 2.5 and 12.7 cm diameter, 38.1 cm above the ground, were felled and the basal 38.1 cm transported to the laboratory where holes were drilled horizontally into the center of the sections 7.6 and 15.2 cm below the surface on one end. Thermistor probes of the same type used in the field studies were placed in these holes and on the surface of the sections. The sections were then placed in a soil box so that the height of the surface was 30.5 cm above the soil level.

In addition to the Fenwal surface probe, a Veco probe was also used to check the reliability of the Fenwal probe. The Veco probe was considerably smaller in size and mass than the Fenwal probe. Radiation errors, using the Veco probe, are small.

RESULTS AND DISCUSSION

Penetration of Stump Tissue by Mycelium of F. annosus

Average downward rates of penetration in stumps at Clallam Bay in 1967 exhibited a seasonal pattern, with rates being highest in summer and lowest in winter to spring. Table 1 shows that average

penetration in cm per week was 0.50 (February-July), 1.49 (May-September), 1.16 (June-October), and 0.59 (September-December). These rates of penetration are of the same order of magnitude as those found by Cowling and Kelman (1964) and Cobb and Barber (1968).

This seasonal pattern of penetration is explainable in terms of the temperature environment at Clallam Bay in 1967. As ambient temperatures approached the optimum temperature growth of F. annosus (25°C) (Table 3), rates of penetration increased. From February to July there were only 12 growing degree days above 15° C base (Table 2), which corresponded to the period of the lowest penetration rate. In contrast, in the period of the greatest penetration rate, 45 growing degree days above 15° C base occurred. As the number of growing degree days above 15°C increased, the rate of penetration also increased, and vice versa.

Penetration rates were not equal in all diameter classes as shown in Table 1. Fomes annosus had the slowest penetration in the smallest diameter stumps from February to July. On the other hand, the fastest penetration occurred in the smallest diameter stumps from May to September. Penetration from September to December was not markedly different between diameter classes.

These results are explicable in terms of stump internal temperatures. Internal stump temperatures at Clallam Bay on August 23 (Table 5) indicated that the smaller diameter stumps reached higher internal temperatures than larger diameter stumps. Temperatures closer to the optimum for maximum growth (25°C) occurred in smaller diameter stumps. Throughout the summer period stump internal temperatures were probably closer to 25° C than those in larger stumps. Thus, greatest penetration occurred in the smallest diameter stumps from May to September.

Although stump temperatures were not measured in winter to spring at Clallam Bay, it would be expected that smaller diameter stumps would be colder than larger, better insulated stumps. This possibly explains the slow penetration in the small diameter stumps from February to May. From September to December, temperatures would have been more favorable for growth in the smaller diameter stumps at the beginning of the period. As ambient temperatures fell, the stump internal temperatures would have quickly become less favorable for growth in the small diameter classes. Table 2 shows the sharp decline in number of growing degree days from September to October. On the other hand, temperatures probably remained more constant in the larger diameter stumps, and, as a result, penetration rates in all diameter classes were similar in this period.

Linear Growth Rates of F. annosus and Associates in Culture

Table 3 shows the linear growth rates in culture of F. annosus isolates from hemlock stumps in the Pacific Northwest. Growth rates for common associates are also shown. All isolates of F. annosus had similar growth rates despite the large geographic distances between sources. The average maximum growth rate was 0.85 cm per day at

25°C. These rates are similar to those found by Gooding, et al. (1966), whose isolate from Pinus taeda also had a maximum at 25°C of 0.9 cm per day. Gooding et al. (1966), however, found that their isolate had a much greater growth rate at 30°C (0.6 cm per day) than any of the isolates from western hemlock.

The most common associates, Graphium sp. and Leptographium sp. had slower growth rates than F. annosus in culture, especially above 10°C.

Thermal Inactivation of Conidia of F. annosus on Stump Surfaces

Inactivation of conidia of F. annosus occurred after one hour at 45° C and between 6 and 12 hours at 40°C, as indicated by inability to germinate at 25°C (Table 4). Ross (1969) also indicated that conidia and basidiospores of F. annosus on loblolly pine stem discs, similarly, were inactivated by 1 hour at 45°C and 90% relative humidity. Germination still occurred after 12 hours at 35°C. Conidia of F. annosus would not germinate at 25°C after 7 days at 0°C. Unfortunately, short periods of exposure to 0°C were not tested, although they may be important. One hundred percent germination occurred after 12 hours at 5°C, but in tests of germination of conidia at continuous temperatures of 5 and 10°C only 20% of the sections showed germinated conidia after two weeks.

Stump Temperatures, Surface Temperatures, and Maximum Temperatures

Although ambient temperatures appear to be directly related to the success of stump infection by F. annosus, it is obviously the temperature of the stump surface which is of direct importance, as it is here that the spore must germinate.

Stump surface temperatures were measured at Clallam Bay in three stumps of different diameters on August 23, 1967. The maximum surface temperature attained was 26.0°C, which was 7°C above ambient. Maximum surface temperatures were 3.5 to 4.0°C hotter on the smaller stumps compared to the 15.2 cm diameter stump (Table 5). The stumps were partially shaded during the day.

Simulated stumps were set up to determine the maximum temperatures that could be obtained on stump surfaces if they were exposed to full sunlight for a maximum period of the day. Figure 2 indicates that on August 26, 1967, surface temperatures on the 2.5 and 12.7 cm diameter stumps reached maximums of 34.0 and 33.5°C, respectively, i.e., 8.0 and 7.5° C above maximum ambient. Surface temperatures estimated by both the Fenwal and Veco thermistors were almost indentical, indicating that the Fenwal probes used at the field sites gave valid estimates of stump surface temperatures -- a difficult parameter to measure.

Evidence from simulated stumps and stumps at Clallam Bay indicated that smaller stumps (2.5 cm diameter) tend to attain slightly higher surface temperatures than larger stumps.

Stump surface temperatures were measured continuously in both western hemlock and Douglas-fir stumps from August 1967 to January 1968, although the data is incomplete due to technical difficulties.

Table 6 indicates that average maximum temperatures on the surface of the Douglas-fir stump at Pack Forest are considerably higher than those of the hemlock, and approach those found one-quarter of an inch below the surface of a pine stump at Durham, North Carolina, for the month of August. Gooding et al.(1966) found an average maximum temperature of 44.1°C for this stump. The absolute maximum temperature recorded for the Douglas-fir stump was 45°C compared to only 24.5°C on the hemlock. The maximum deviation above maximum ambient was 16.4°C for the Douglas-fir stump and only 9°C for the hemlock. The results for the hemlock stump agree well in magnitude with the data from the simulated stumps.

These data suggest that there was a difference in the thermal properties of the two stumps. However, the data from Cedar River show that high temperatures were not attained on Douglas-fir stumps. The data used, however, are poor and no conclusion should be drawn from them. There is a need for future work to determine if there is a real difference between the species.

The maximum surface temperature recorded on a hemlock stump was 34.5°C, which would not be hot enough to inactivate spores of F. annosus.

Table 4 shows that conidia were able to germinate at 25°C after 12 hours at 35°C. These results indicate that exposure time at a given temperature is important. With this in mind, the number of hours per day between 5°C limits was calculated for the hemlock stump at Pack Forest in September. An average of only 0.46 hours per day fell between 30-35°C, whereas 20.86 hours per day fell between 5 and 20°C (Figure 3). Thus, factors other than maximum temperatures must be considered in inactivation of spores on western hemlock stumps.

Schmidt and Wood (1969) working with pine stumps in Pennsylvania also found that stump surface temperatures were near optimum in August except for a few period when temperatures above 32°C were recorded.

Minimum Temperatures

Stump surface temperatures in the colder months were found to vary little from ambient (Table 6). Mean minimum surface temperature of the Douglas-fir stump in the clearcut was -2.4°C and, of that in the stand, -0.8°C for January 1968. A majority of the hours per day (15.9) was less than 0°C on the surface of the stump in the clearcut, and 21.1 hours per day were less than 5°C (Figure 4). This agrees with the number of hours per day occupied below 5°C on the hemlock stump surfaces at Pack Forest (17.7).

Conidia of F. annosus would not germinate at 25°C after 7 days at 0°C (Table 4). Conidia withstood 5°C for 12 hours, but in tests of germination at 5 and 10°C continously, germination was poor, with only 20% of the discs showing germinated conidia.

Thus, in some of the colder areas of the western hemlock region, minimum stump surface temperatures could be limiting to the development of F. annosus.

Stump Internal Temperatures

Gooding, et al. (1966) found that two hours at 40°C killed actively growing mycelium of F. annosus in wood chips, but mycelium could survive 8 days at 35°C. Stump internal temperatures were measured in western hemlock and Douglas-fir stumps to determine if the lethal limit could be attained.

The maximum internal temperature recorded in western hemlock stumps was 37.3°C in the 2.5 cm simulated stump (Figure 2), and smaller diameter stumps tended to attain higher maximums than larger stumps (Table 5, Figure 2). Internal temperatures exceeded surface temperatures in simulated stumps at Clallam Bay because of lateral heating of the stumps.

Average monthly maximum and minimum internal temperatures for hemlock and Douglas-fir stumps at Pack Forest and Cedar River are given in Tables 7 and 9. Temperatures approached the lethal range only in the Douglas-fir stump at Pack Forest (Table 7) in August.

However, Gooding et al. (1966) illustrated that the number of hours at a given temperature is as important to the actual maximum in thermally inactivating mycelium. Figure 3 shows that only .01 hours per day was spent between 30 and 35°C in the hemlock stump at Pack Forest in September. Most hours of the day were occupied between 5 and 20°C. Thus it would appear that lethal maximum temperatures for inactivation of mycelium would rarely be approached.

Actual lethal minimum temperatures for mycelium of F. annosus have not been reported, but growth is negligible at 0°C. Tables 7 and 9 illustrate that mean monthly minimum internal temperatures rarely fall below 0°C. However, Figure 4 illustrates that many hours of the day can be occupied less than 0°C. Internal stump temperatures in winter months will certainly inhibit growth of F. annosus in stumps.

CONCLUSIONS

Penetration of western hemlock stumps by F. annosus at Clallam Bay followed a seasonal trend with the greatest penetration occurring in summer. Penetration was not equal in stumps of all diameters. The penetration pattern is related to the temperature environment, particularly stump temperatures. Small diameter stumps were more sensitive to temperature changes.

All isolates of F. annosus from western hemlock stumps had a maximum linear growth rate at 25°C and grew poorly at 0 and 30°C. Of the three associates also tested, only Trichoderma sp. exhibited better growth rate. Trichoderma sp. was observed only rarely.

Investigations into stump surface and internal temperatures, combined with conidial germination studies, have indicated that, in general,

the temperature environment is suitable for the germination of F. annosus spores and peneteration of mycelium into western hemlock stumps throughout most of the year. Evidence would indicate that F. annosus is more likely to be limited by cold stump temperatures than hot.

The apparent lack of effective competitors, combined with the generally favorable stump temperature environment, would indicate that the likelihood of seasonal control of F. annosus stump infection, as occurs in southeastern U.S.A., (Ross, 1973), is remote in the western hemlock region.

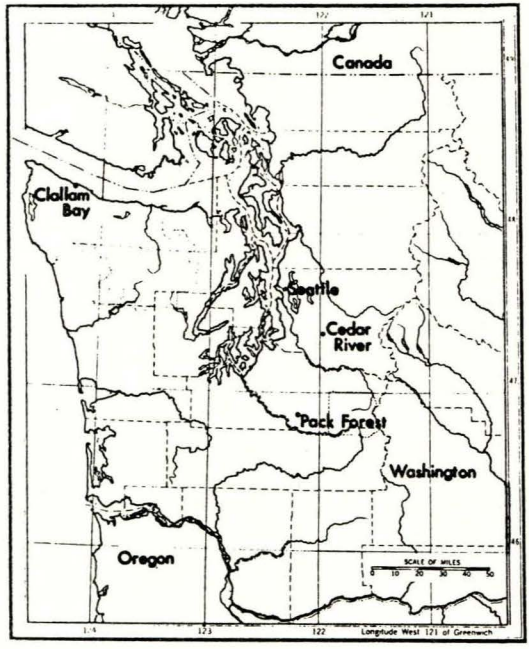

Figure 1. Location of research areas in western Washington

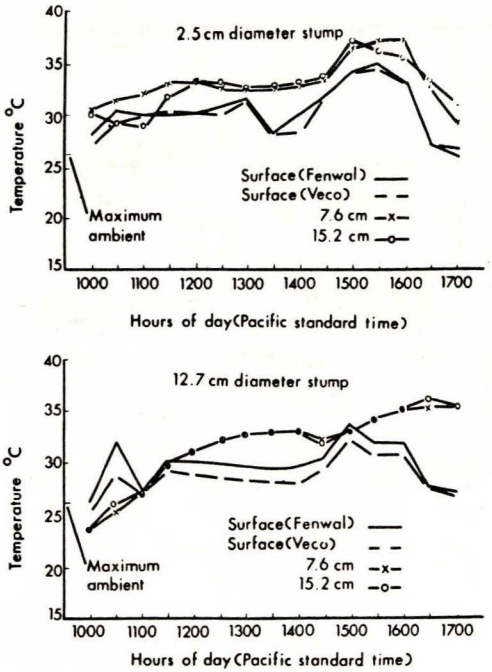

Figure 2. Stump temperatures for two simulated stumps at the surface and internally 7.6 and 15.2 cm below the surface at Seattle, Washington, August 26, 1967. Surface temperatures were measured with two different types of thermistor probes.

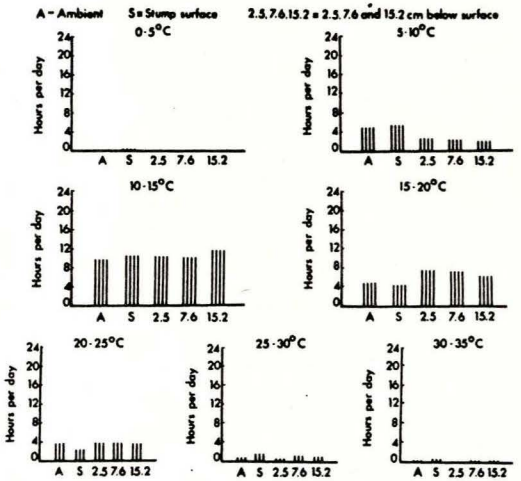

Figure 3. The average number of hours per day occurring in the ranges from 0-5, 5-10° C, etc., in September, 1967 for ambient temperatures, and the temperatures of a 14.7 cm diameter western hemlock stump at the surface and internally 2.5, 7.6 and 15.2 cm below the surface.

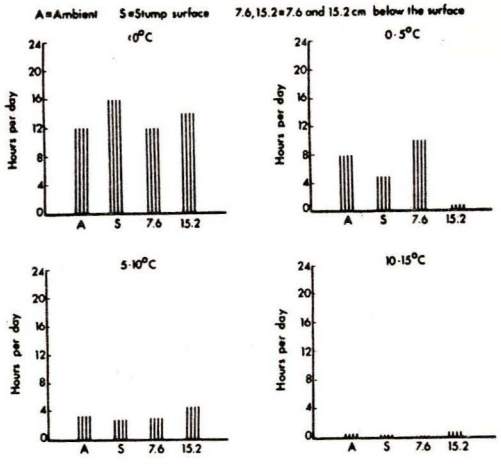

Figure 4. The average number of hours per day occurring in the ranges, <0, 0-5, 5-10, and 10-15° C in January, 1968 for ambient temperatures, and the temperatures of a 21.6 cm diameter Douglas-fir stump at the surface and internally 7.6 and 15.2 cm below the surface.

Table 1. Rates of penetration of mycelium of Fomes annosus in naturally infected western hemlock stumps, by diameter classes, exposed after thinning in February, May, June and September 1967, at Clallam Bay, Washington.

Month of Stump Exposure	Month of Sampling	Penetration (cm per week) Diameter Classes (cm)			
		0.0-3.7	3.8-6.3	> .4	Average
February	July	0.30	0.57	0.60	0.50
May	September	2.13	1.59	1.25	1.49
June	October				1.16
September	December	0.64	0.46	0.63	0.59

Table 2. Growing degree days* at Clallam Bay, Washington, in 1967 above a base of 15°C.

Months	15°C
January	0
February	0
March	0
April	0
May	1
June	3
July	8
August	11
September	22
October	0
November	0
December	0

* Growing degree days are defined as the difference between the mean temperature for the day and 15°C.

Table 3. Linear growth rates in culture from 0-30°C of *Fomes annosus*. *Graphium* sp., *Leptographium* sp., and *Trichoderma* sp. isolated from western hemlock stumps.

Species	Location of Isolate	Growth rate (cm per day) Temperature (degrees C)						
		0	5	10	15	20	25	30
F. annosus	Clallam Bay, Washington	0.01	0.02	0.33	0.60	0.73	0.98	0.00
F. annosus	Clallam Bay, Washington	0.03	0.19	0.28	0.60	0.75	0.86	0.03
F. annosus	Clallam Bay, Washington	0.01	0.12	0.27	0.49	0.57	0.61	0.00
F. annosus	Clallam Bay, Washington	0.02	0.15	0.32	0.63	0.73	0.86	0.00
F. annosus	Montesano, Washington	--	0.00	0.20	0.45	0.65	0.98	0.13
F. annosus	Cathlamet, Washington	--	0.04	0.29	0.57	0.61	0.91	0.13
F. annosus	Seaside, Oregon	--	0.14	0.23	0.22	0.63	0.75	0.00
Average all locations		0.02	0.09	0.27	0.51	0.67	0.85	0.05
Trichoderma sp.	Clallam Bay, Washington	--	0.09	0.22	0.86	1.85	2.45	3.22
Graphium sp.	Clallam Bay, Washington	--	0.00	0.12	0.14	0.15	0.28	0.12
Leptographium sp.	Clallam Bay, Washington	--	0.11	0.14	0.14	0.42	0.60	0.00

Table 4. The effect of temperature on the ability of conidia of Fomes annosus to germinate at 25°C on western hemlock stem sections.

Initial Temperature (Degrees C)	No. of Hours at Initial Temperature Before Transfer to 25°C	No. of Sections Upon Which Germination Occurred	Total No. of Sections	Percent of Sections Upon Which Germination Occurred
0	168 (7 days)	0	5	0
5	1	5	5	100
	6	5	5	100
	12	5	5	100
10	1	5	5	100
	6	5	5	100
	12	5	5	100
25		5	5	100
30	1	5	5	100
	6	5	5	100
	12	5	5	100
35	1	5	5	100
	6	5	5	100
	12	5	5	100
40	1	5	5	100
	6	4	5	80
	12	0	5	0
45	1	0	5	0

Table 5. Maximum temperatures on the surface, 7.6 and 15.2 cm below the surface of 2.5, 4.8, and 15.2 cm diameter western hemlock stumps on August 23, 1967, at Clallam Bay, Washington.

	MAXIMUM TEMPERATURES °C			
Stump Diameter (cm)	Ambient	Surface	7.6 cm	15.2 cm
	19.0			
2.5		25.5	25.5	27.5
4.8		26.0	24.0	26.0
15.2		22.0	18.0	18.0

Table 6. Average monthly maximum and minimum temperatures of surfaces of western hemlock and Douglas-fir stumps at two locations in western Washington.

	TEMPERATURES °C					
Month	Ambient	Douglas-fir	Western Hemlock	Ambient	Douglas-fir Clearcut	Douglas fir Stand
August 1967						
Average Max.	30.6	41.9	--	--	--	--
Average Min.	12.3	14.8	--	--	--	--
September 1967						
Average Max.	23.8	31.5	26.8	15.8*	22.0*	19.9*
Average Min.	10.3	11.0	9.0	9.7*	10.6*	17.0*
October 1967						
Average Max.	14.1	16.8	16.7	15.8	--	--
Average Min.	7.4	6.9	8.7	5.5	--	--
November 1967						
Average Max.	9.0	10.9	10.5	9.6	7.3	6.3
Average Min.	5.3	6.4	5.8	2.0	0.0	1.5
December 1967						
Average Max.	6.8	3.0	2.6	4.2	1.1	2.2
Average Min	1.4	--	--	-2.6	-1.6	0.5
January 1968						
Average Max.	7.4	--	--	3.1	1.7	2.1
Average Min.	0.8	--	--	-0.8	-2.4	-0.8

*Estimated from only 8 days data.

Table 7. Average monthly maximum and minimum temperatures in western hemlock and Douglas-fir stumps, at Pack Forest, Washington, 2.5, 7.6, 15.2 cm below the stump surface.

T E M P E R A T U R E S ° C

	Douglas-fir			Western Hemlock		
Month	2.5 cm	7.6 cm	15.2 cm	2.5 cm	7.6 cm	15.2 cm
August 1967						
Average Max.	36.8	36.2	34.9	--	--	--
Average Min.	14.9	17.2	17.4	--	--	--
September 1967						
Average Max.	29.7	--	28.9	22.5	23.8	22.5
Average Min.	10.5	--	11.3	10.8	11.1	8.1
October 1967						
Average Max.	16.1	17.6	--	17.1	16.6	16.4
Average Min.	7.4	9.0	--	9.4	9.6	10.2
November 1967						
Average Max.	10.1	11.6	--	9.3	9.1	9.7
Average Min.	5.7	6.7	--	5.9	5.7	5.9
December 1967						
Average Max.	--	--	--	--	--	--
Average Min.	--	--	--	--	--	--
January 1968						
Average Max.	--	--	--	3.5	3.7	3.6
Average Min.	--	--	--	--	--	--

Table 8. Average monthly maximum and minimum temperatures in Douglas-fir stumps at Cedar River, Washington, 7.6 and 15.2 cm below the stump surface.

TEMPERATURES °C

	Stump in Clearcut		Stump in Stand	
Month	7.6 cm	15.2 cm	7.6 cm	15.2 cm
September 1967				
Average Max.	18.6	19.1	15.9	15.7
Average Min.	13.8	15.7	14.5	5.1
October 1967				
Average Max.	--	--	--	--
Average Min.	--	--	--	--
November 1967				
Average Max.	5.8	7.5	7.8	7.0
Average Min.	2.3	4.9	5.6	5.5
December 1967				
Average Max.	1.6	3.5	4.7	3.7
Average Min.	-0.2	2.1	2.2	2.7
January 1968				
Average Max.	1.6	3.5	3.6	3.6
Average Min.	-0.6	1.7	1.9	2.5

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DISCUSSION ON

EFFECT OF EDAPHIC AND ENVIRONMENTAL FACTORS

Eldon Ross, Chairman

Kuhlman: Presented paper entitled "Variation in infection of loblolly pine roots on high and low hazard sites in the southeastern United States."

DISCUSSION

Sylvestre: What is the effect of the water table on rate of decay?

Kuhlman: On the low hazard site with the high water table the decay rate is slowed (probably because of a reduced 0_2 level) over the rate on high hazard sites. We had thought that this slower rate might have permitted infection of the residual tree roots but only a limited spread. Actually, we found less infection.

Alcubilla: What do you feel is the effect of nutrition on infection and disease development?

Kuhlman: I don't believe host nutrition affected disease development since both stands had good growth. We did not determine the nutrient makeup of the trees in either stand.

Hodges: Did you find differences in rooting patterns in high and low hazard sites?

Kuhlman: Yes, on low hazard sites the roots tend to branch quickly and there are more roots growing downward from the laterals.

Krebill: How do you account for the difference in symptom expression and isolation of the fungus?

Kuhlman: These examinations were made 4 and 6 years after thinning and some of the infections were probably sealed off and the fungus had died. Other symptoms might not have been due to F. annosus.

Skelley: How can you be sure this ectotrophic growth of F. annosus on roots is not the result of percolating spores?

Kuhlman: Four to 6 years after the stumps were created it is impossible to determine the courts of infection. Our experience suggests less than 10% of stump root infections are the result of spores in the soil.

Rishbeth: In this investigation the proportion of observed root contacts that resulted in infection was 4.3 times greater on high hazard sites than on low hazard ones. This is comparable with the situation found earlier in eastern England although there the root systems of the pines were very similar on the contrasted sites and the main difference between them was soil pH.

Wallis: What was the pattern of mycelial development on roots on low vs. high hazard sites?

Hodges: Surface development of mycelium was found in bark scales on high hazard sites. Biological and/or physical factors at contact point on low hazard sites inhibit spread from stump roots to roots of residual trees.

Wallis: Did you isolate from point of contact at stump root with residual tree root?

Kuhlman: No, we tried to confine our isolations to roots which appeared to have decay which looked like F. annosus.

Cobb: It seems that roots in sandy soils would be more apt to occur at a greater depth than those on poorly drained clays with a high water table. Why is this not the case?

Hodges: Roots in clay soils rapidly decrease in diameter as they move away from the tree, and after only a short distance they branch into a number of small roots that grow sharply down to lower levels.

DISCUSSION

Alexander: Presented paper entitled "Disease incidence and disease severity in loblolly pine planted over two soil hazard types."

Shain: Can you go into the field and identify a high and low hazard site based on soil texture?

Alexander: Yes. In Virginia. I can say the possibility is high that F. annosus is or is not there. From the standpoint of mortality I think you can use soil factors such as sand content, clay content, and depth of water table at 20-30 cm depth, but from the standpoint of growth loss, if the data continues to hold up, soil factors don't seem to be related.

Hodges: In terms of making isolations from whole root systems, was F. annosus present in your isolations only from areas which showed some recognizable visual symptoms? Is it necessary to do a lot of isolations or, do you think you could make isolations only from suspected areas and still come out with the right percentage of infection?

Alexander: Most of the time, looking only at symptoms, you could get a pretty good idea whether F. annosus was there. However, the last 3 years I have isolated from almost 500 trees, 2 roots from each tree, 1000 roots. I found many roots that had no outward symptoms but I was able to isolate F. annosus from them. Generally speaking I would say that you could rely on symptoms, particularly if its a stump root.

Froelich: If you work within a limited area you can develop certain correlations, but as you expand your area of operation from two side by side plantations to a state or several states, a nation, to the world, your correlations are going to break down. You have to speak of specific areas, for example, I would have to agree with the work of Morris and Frazier and their criteria in general for rating sites, depth of water table, being a very important variable, and of course deep sands are very important. There are some very notable exceptions in our Gulf south area of Mississippi where we have wind blown soils, called loess. These are very deep soils probably over 50% silt and as far as I can determine they are just as high a hazard as any deep sand. There are occasional very high clay (20-30%) sites where clay is right at the surface and these can be at least moderate hazard sites. Only time will tell whether you could move this rating up to high hazard and what the future impact of the disease will be. Our experience so far is that you get rapid killing the first few years after thinning, then mortality stabilizes, but we don't know if later on in the life of the stand there will be increased mortality and windthrow?

Wallis: What is the pattern of fungus development in isolated low hazard areas? How do you explain the 40% infection on the low hazard sites if you don't get any mortality?

Alexander: The only answer that I can give is that the tree is less apt to be under moisture stress than a tree on a sandy site, and the host defense mechanisms on low hazard sites appear to be much stronger than on high hazard sites.

Skelley: Don't confuse incidence of infection with severity, there is up to 40% incidence, on these low hazard sites, but the severity of infection can be determined only by excavating the entire root system and rating it.

Wallis: Yes, I understand that, but I wanted to know why this happens?

Skelley: From the data we have collected, it appears to be more a matter of how much inoculum reaches the root surface rather than a resistance mechanism in the tree. It becomes more a matter of inoculum potential either through root contacts or spore percolation. Of course there are many soil parameters that could affect subsequent growth, but first the spores must get to the root surface.

Hodges: Dr. Ross and I have completed a very large study conducted over the entire southeastern United States. We studied the influence of soil type on the actual spread of F. annosus from known infection centers. We established a large number of artificial infection centers and on the basis of our studies, in general, we can say there is a difference in the actual rate of spread in soils of different types. The difference in rate of spread was better than 10m in 5 years on high hazard sites and to as low as 2-3 meters in 5 years on lowest hazard sites. The biggest problem of course is all those in between the spread of 4-5 m in 5 years, it is very difficult to say whether these are high, low or even if the rate of spread correlates with soil factors. But in general, I think we would have to agree with previous work that extremely deep sands, well drained sites, are high hazard sites and in general heavy clay soils or sandy soils with high water tables are low hazard sites, but it is very difficult to define the middle.

THE INFLUENCE OF SOIL BIOLOGICAL FACTORS ON THE MYCELIAL GROWTH OF *FOMES ANNOSUS*

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INTRODUCTION

Although *Fomes annosus* (Fr.) Cke. is thought to spread from tree to tree through root contact, several investigators have suggested that the fungus may also spread locally by growing from an infected tree through the soil to infect adjacent trees. However, when they investigated the possibility of local spread through the soil, their studies showed that *F. annosus* was inhibited by some soil inhabitants (4,6,11). Nevertheless, there were certain results from these and other investigations which indicated that under conditions of low temperature and/or reduced microbial populations the fungus could possibly grow through the soil (1,4,8,9,12).

The study described here was designed to ascertain if growth of *F. annosus* through soil could be a factor in local spread in some soils of the Sierra Nevada Mountains in California. The specific objectives were to: (a) determine the conditions under which *F. annosus* might grow in the soil, and (b) determine the influence of soil biological factors on the growth of *F. annosus* in soil.

INFLUENCE OF SOIL MICROBIAL POPULATIONS

The capacity of F. annosus to penetrate into and colonize field soil appears to be greatly influenced by the presence or absence of soil antagonists. An estimate of the effect of these antagonists should be obtainable through various means of manipulating the total soil population.

Two techniques were used to test this approach: (a) determining the growth of F. annosus in soil collected at different soil depths where the microbial populations would vary, and (b) determining the growth of F. annosus in soil pasteurized at various temperatures to gradually reduce the numbers and types of microorganisms present in the soil.

Methods and Materials

Soil depth studies: Soil was collected from six plots on the Blodgett Experimental Forest, which is located at 4500 feet elevation in a mixed conifer forest in the central Sierra Nevada Mountain Range.

The soil samples were collected at depths of 3, 15, 31, 41, 61, 76, 81 and 107 cm on all plots except 5 and 6 where soil was collected to only 76 cm. These samples were divided and used to determine soil microbial populations and the rate of growth of F. annosus.

In the growth studies, an inoculum chip which was a 2.54 cm^2 piece of ponderosa pine infested with F. annosus was buried in the soil in petri dishes and incubated at 15°C for seven days. At the end of the incubation period the growth of F. annosus in the soil was measured.

Population counts of the microflora found in the soil were obtained by plating out $1:10^4$ soil dilutions on three media: Rose Bengal for fungal counts and soil extract agar plus peptone or glucose for actinomycetes and bacteria.

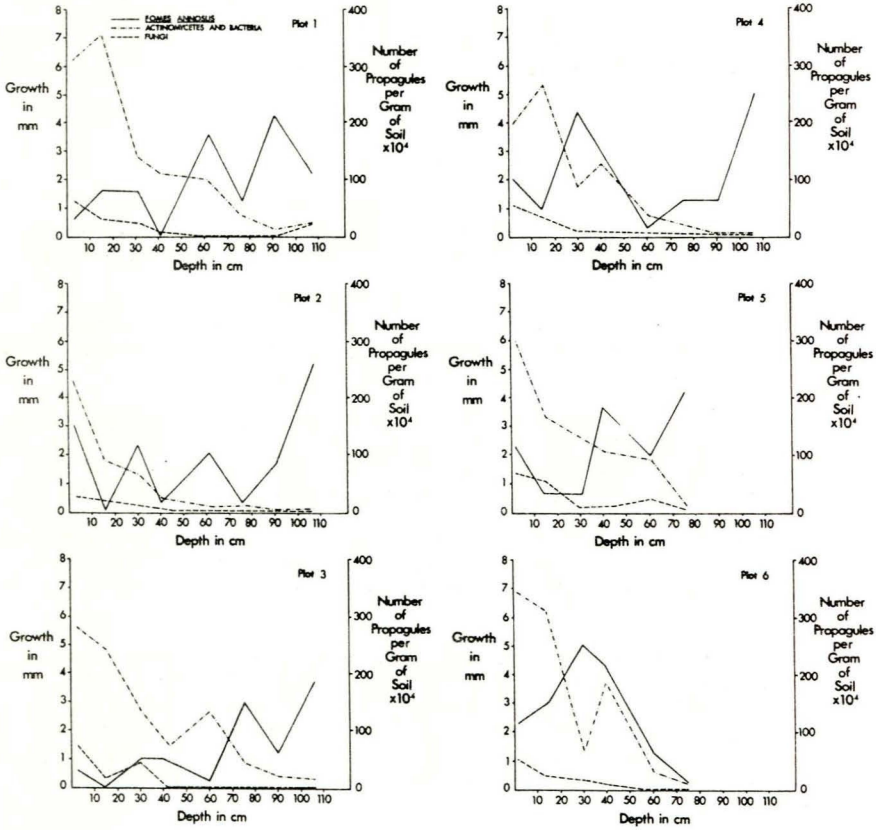
Soil pasteurization: This method utilized a steam pasteurization apparatus designed by Baker (2). Soils were stream treated at 140°, 160°, 180°, 200°, and 212°F for one-half hour. The same method to determine growth rate in the soil depth studies was used to test the ability of F. annosus to grow through the pasteurized soil. Microbial population counts were obtained for the soils treated at 180°, 200°, and 212°F.

Results

There was an apparent relationship between F. annosus growth and the level of microbial soil populations. The graphs in Figure 1 show a general trend toward increased growth rate with a reduction in the number of organisms per gram of soil. However, rather than showing a gradual increase in growth through the soil as the microbial populations decline, growth appeared to fluctuate widely and in plot 6 the growth

of F. annosus declined at approximately the same rate as the microbial population.

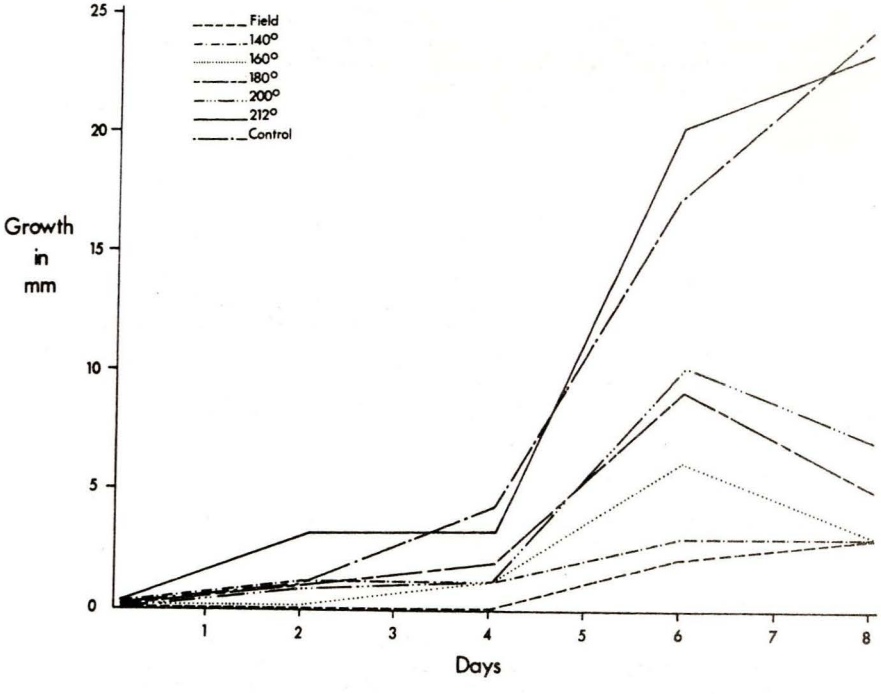
Figure 1.--The influence of microbial populations on the growth of Fomes annosus in the soil



When the data was analysed, the F values were significant at the five percent level of probability; however, the coefficient of multiple correlations showed that the regression model explains only 1 to 4 percent of the variation. Therefore, the relationship between F. annosus growth and level of soil populations is probably weak and can only be detected with large numbers of observations.

In the pasteurization experiments, there was an almost direct relation between the temperature at which the soil was treated and the rate of linear growth of F. annosus through the soil (Figure 2). The fungus grew 6, 9 and 10mm in soils treated at 160°, 180°, and 200°F respectively in six days. The best growth was in soil treated at 212°F. F. annosus grew as well in soil treated at this temperature as in the sterilized, control soil, 23 and 24mm respectively in an eight-day period.

Figure 2.--Rate of growth of Fomes annosus in soils pasteurized at different temperatures



The microbial numbers per gram of soil at 180° and 200°F were comparable to the population at 31 cm in the soil depth studies, but the growth of F. annosus in the pasteurized soil was always more than that in the soil depth experiments where the microbial population levels were similar. The evidence would indicate that there is either a more complex relationship than that involving number of propagules per gram of soil or that other factors, either singly or cumulatively, have a greater effect than the general level of microbial populations on the growth of F. annosus in the soil.

In all the experiments involving field soil, F. annosus would occasionally form mycelial strands. The strands appeared to be intertwining hyphae without the differentiated areas found in the rhizomorphs of Armillaria mellea. These strands grew as much as 12-15 mm in field soil in seven days, a rate much greater than that of any single hypha. The mycelial strands did not form in soils that had been pasteurized or sterilized.

SOIL VOLATILES AND BY-PRODUCTS

Results of the above studies indicate that reduction of soil microbial populations through various manipulations had a relatively small effect upon growth of F. annosus unless these populations were almost totally eliminated. Perhaps volatile compounds and other by-products produced by a relatively few microbes in the soil and duff can influence F. annosus growth. The following experiments were designed to determine if such products are present.

Methods and Materials

Duff, humus and soil to a depth of 61 cm were collected on the area around plot 6. Plot 6 soil was used because growth decreased even with a decrease in microbial populations. The material was divided into the following levels: (a) duff and humus, (b) soil 0-15 cm from the surface, (c) 15-31 cm from the surface, (d) 31-46 cm from the surface, and (e) 46-61 cm from the surface. Vacuum desiccators were filled 3/4 full with material from each of the different levels. Ten 9 cm petri dishes containing water agar were inoculated with a piece of F. annosus mycelium and placed in each desiccator. Each desiccator represented one treatment. Linear growth was measured every five days for a 15-day period. The control was the same as described above except that the desiccators contained no duff, humus or soil.

The effects of cell-free soil extracts were studied also. In these experiments field soil from plot 6 and sterile water were mixed in equal parts in a 2 liter erlenmeyer flask and allowed to stand for 24 hours. The supernatant was collected and filtered through #2 Watman paper. Next, it was centrifuged at 18000 rpm for 15 minutes and then filtered through a millipore filter.

The cell-free soil extract was added to water agar at cencentrations of 50, 100, 200, 400, 800, 1,000, 5,000, 10,000, and 100,000 ppm.

Pieces of F. annosus mycelium growing on PDA were cut with a #2 cork borer and placed on this agar at one edge. The control was water agar with no additives.

Results

Within the volatile experiments using the vacuum desiccators there were indications that in the duff and some levels of soil the volatiles were inhibitory or stimulatory on the growth of F. annosus.

Blodgett Experimental Forest has distinct wet and dry seasons and samples were collected during both seasons. When the samples were collected during the wet season volatiles from the duff and humus layers were very inhibitory to the growth of the fungus and this inhibition at times extended into the top 15 cm of field soil; e.g., in one wet treatment F. annosus grew only 11 mm in the presence of duff, while the control grew 62 mm, in a 15-day period.

When the samples were collected during the dry season, volatiles from duff and soil at times stimulated the growth of F. annosus. Though the results were not consistent, the growth of the fungus was never inhibited by samples collected during the dry season.

In the cell-free soil extract experiments there appeared to be an inverse relationship between the concentration of cell-free soil extract and the rate of growth of F. annosus (Table 1). Analysis for variability among treatments using a one-way analysis of variance yielded an F value of 10.9482 (significant at .01 level). Treatment means were compared using Tukey's W for comparative means. On media containing concentrations of 5,000, 10,000 and 100,000 ppm of cell-free soil extract the fungus grew significantly less than the control.

Table 1.--Effects of cell-free soil extract on *Fomes annosus* growth[a]

200 ppm	Control	400 ppm	100 ppm	800 ppm	50 ppm	1000 ppm	10000 ppm	5000 ppm	100000 ppm
- X	- X	- X	- X	- X	- X	- X	- X	- X	- X
42.37mm	42.0mm	40.68mm	39.11mm	38.40mm	37.75mm	37.0mm	36.29mm	34.14mm	31.57mm

a Growth means with a common line are not significant.

LYSIS OF FOMES ANNOSUS IN SOIL

Experiments were designed to determine what happens to the mycelium of F. annosus in the soil and also to determine if it can exist in the soil for an extended period of time.

Methods and Materials

F. annosus was grown on untreated cellophane on water agar in petri dishes. When the mycelium covered three quarters of the cellophane surface, the cellophane with the mycelium was removed from the water agar and buried in field soil at 15°C for 24, 48, and 72 hours.

The mycelium was then removed from the soil, washed with tap water and examined for lysis or other disruptions of the hyphae. The controls were treated the same way, with the exception that sterile soil was used in place of field soil.

Results

The mycelium that had been in the soil 24 hours had no observable changes in the cell wall structure. After 48 hours some of the hyphal cells had lysed and in other instances there appeared to be leakage of intracellular material through the cell wall which caused soil particles and some other soil inclusions to adhere to the cell wall. After 72 hours there was almost a complete breakdown of the mycelium.

Motile bacteria were associated with the hyphal cells that were lysed and with those that accumulated soil particles on the cell wall surfaces. It was not determined whether the bacteria were causing the lysis or were being attracted to the areas by the release of intracellular materials.

The control showed no signs of lysis or accumulation of materials around the cell walls.

DISCUSSION AND CONCLUSIONS

The results of these studies indicate that F. annosus will grow at least short distances through field soil. However, they showed that F. annosus is dramatically inhibited in the soils tested, apparently at least in part by antagonistic soil microorganisms. These results tend to support the work of Treschow (11), Rennerfelt (8), Francke-Grossman (5), Bjorkman (3), Nissen (7) and many others who demonstrated that certain soil inhabitants are antagonistic to F. annosus in vitro.

There are indications that volatiles and other by-products in the soil influence the growth of F. annosus and that under some circumstances inhibition may be as great as that observed when F. annosus was placed in field soil.

It is also apparent from the studies of lysis that mycelium of F. annosus cannot exist in the soil for any extended period of time, which precludes the possibility of it being a soil inhabitant. However, the fungus does infrequently form mycelial strands which grow at a greater rate than simple hyphae. The strands are also probably more resistant to antagomistic soil inhabitants and may have a role in local spread.

In conclusion, there is some evidence that F. annosus may have the limited ability to spread through the soil to attack adjacent trees. This type of spread is probably rare and would possibly only function when roots are in proximity, as was found in the field by Towers (10). F. annosus is not a soil inhabitant and could not function as one unless through some disaster of nature the soil became almost devoid of other soil microorganisms.

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STUDIES ON FOMES ANNOSUS AND THE INFECTION OF PINE SEEDLINGS[1/]

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A reliable method of inoculating individual trees in the forest is needed for the conduct of controlled research on root and butt rot of pine caused by Fomes annosus (Fr.) Karst. No inoculations with this fungus in the forests at Clemson, S. C. have resulted in mortality due to infection. In 1969, Kuhlman (4) described a method whereby seedlings in the greenhouse could be successfully inoculated using fungus-infested beech blocks. During May and June of 1970, a series of inoculations in an air-conditioned greenhouse was made using Kuhlman's method. Of 134 trees inoculated, 61 were killed and F. annosus was isolated from 40 dead trees. Four of the 134 controls died but no F. annosus was isolated from them. Similar tests conducted in the next 2 months resulted in few deaths, and approximately the same number of inoculated and control trees died. Because the inoculum used in the previous tests was grown in flasks sealed with plastic bags to prevent infestation with fungus mites, the inoculum was considered as a possible cause of failure of

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the fungus to kill the trees. However, when sealed and unsealed inoculum was used in further tests there was no significant difference in the number of trees killed (unpublished data). In further attempts to produce mortality of pines with F. annosus, trees were potted in soil, river sand, washed sand and perlite. Soil in these studies was unsatisfactory as a potting medium (unpublished data). Even in washed sand less than 50 percent of the trees were killed. Variability in inoculum virulence was considered and 19 isolates were used to inoculate 95 seedlings (5 seedlings per isolate). The isolates appeared to vary in virulence and an isolate (NC-128) from Dr. Kuhlman appeared to be slightly more virulent than isolates previously used (unpublished data). Mortality in a subsequent test was still only about 50 percent and in the open pots in the greenhouse the presence of microorganisms was considered as a possible reason for the variability of mortality in various tests. Huppel reported that F. annosus inoculation of Pinus sylvestris L. and Picea abies Karst. in axenic conditions resulted in mortality (1). It was decided that a closed system would give more easily interpreted results. Because of this the following test tube studies were established to determine the effects of medium and amounts of inoculum on infection, and if trees inoculated with different isolates grown on different media would differ in photosynthesis. Respiration rate and catalase activity of the isolates were also measured.

MATERIALS AND METHODS

In a 2x2x3 factorial experiment with 5 replications, surface-sterilization of seed was studied in combination with sterilization of the planting medium. Shortleaf pine (Pinus echinata Mill.) seeds were divided into two equal lots. One lot was surface-sterilized with 33 percent H_2O_2 for 10 minutes. The other lot was not surface-sterilized. Fifteen seeds of each lot were planted in 1"x8" screw cap test tubes containing 3 inches of sand, 3 inches of sawdust, or paper towelling saturated with water. The tubes and media were previously autoclaved at 15 psi for 30 minutes. The test was repeated with seeds treated the same but tubes and media not sterilized. All tubes were placed in a Sherer-Gillette growth chamber set for a simulated 10-hour day (29°C) and a 14-hour night (14.5°C). Six weeks after germination, 3 seedlings in each of 5 tubes were inoculated at 4-week intervals by placing a 3/8-inch square of F. annosus on malt agar against the root. Inoculations continued for 5 months.

To determine the effect of massive amounts of inoculum, the roots of shortleaf pine seedlings (5 per treatment) were enfolded with PDA (potato dextrose agar, MA (malt agar), and K-H (Kuhlman-Hendrix agar (3)) cultures of F. annosus as taken from Petri dishes. Twenty-five slash pine (Pinus elliotti Englm.) seedlings, 1 month old, and 25 shortleaf pine seedlings 4 months old, were placed in 1"x8" test tubes and 5 of each inoculated with two isolates of F. annosus (SRP 522 and NC 128) grown on PDA and K-H. After 3 weeks' growth in the laboratory all seedlings were placed in the Sherer-Gillette growth chamber. The test tubes were connected to a Zollinhoffer closed manometer system (2) and photosynthesis measurements made for 6 hours.

To compare the effects of isolate and growth medium on respiration rate and catalase reaction of F. annosus, 1.75 sq. cm. discs were cut from PDA, MA, and K-H cultures of F. annosus and placed in the Zollinhoffer manometer.

RESULTS AND DISCUSSION

Within 1 week after inoculation, 3 shortleaf pine seedlings in the tubes of sand, sawdust, and paper towelling were dead, and within 8 months, all inoculated seedlings had died. F. annosus was isolated from 60 percent of the dead seedlings. Four of the 150 controls died. Death of these trees may have been related to growth of algae which over-grew the medium.

Three days after roots of shortleaf pine seedlings were enfolded in entire plate cultures, 2 seedlings were dead. After 6 weeks, 7 of the inoculated seedlings were dead and the rest were either yellow or dark purple as compared with the green controls.

Seedlings inoculated with SRP 522 isolate produced significantly more O_2 than did those inoculated with NC 128. None of the seedlings in this study was dead after 3 weeks but those inoculated with F. annosus from K-H medium or with isolate NC 128 were yellow. After 6 weeks 3 controls and 6 seedlings inoculated with NC 128 on K-H medium were dead.

Table 1.--Respiration rates and catalase reaction of two Fomes annosus isolates (NC-128 and SRP-522) on three media.

Isolate	Respiration			Catalase		
	PDA	Malt	K-H	PDA	Malt	K-H
NC-128	0.103[1]	0.070	0.116	2.932	0.643	8.641
SRP-522	0.004	0.002	0.005	2.260	1.350	0.896

[1]Means of 25 samples, rates expressed in ml of oxygen/hr.

Respiration rates between the isolates were significantly different at the 1 percent level, Table 1. Catalase activity between isolates was significantly different at the 5 percent level.

CONCLUSIONS

With massive inoculum some seedlings in test tubes can be killed in a very short time. There is a differential effect of the inoculum on oxygen production during photosynthesis of seedlings because of the isolate used. The respiration rate and catalase reaction of F. annosus varied with the isolate used. Photosynthesis, respiration and catalase reaction tests may prove useful for selecting more active and possibly more virulent isolates.

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INFLUENCE OF ROOT EXUDATES OF RED PINE ON THE GERMINATION OF CONIDIA OF *FOMES ANNOSUS*

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SUMMARY

Root exudates were collected during October from the healthy tips of woody roots of mature red pine in south-central Connecticut. The ability of these exudates to stimulate the conidia of *Fomes annosus* to germinate was evaluated *in vitro* using the silia gel diffusion test and in soil using the buried membrane filter method. Conidial germination was enhanced by root exudates only 3 to 5 percent on silica gel and approximately 1 percent in soil over distilled water controls. It is suggested that conidia in the soil induced to germinate by root exudates may possibly cause direct infection of healthy root tips but that this process is probably sufficiently rare as to be relatively insignificant.

INTRODUCTION

There is considerable evidence to suggest that conidia have importance in the ecology of *Fomes annosus* (Fr.) Cooke. These spores (hyaline, 1- celled, globose to ovoid), while not readily seen in nature, have been observed in underground cavities, on infected stump

wood, in pine root bark, and in various insect galleries and excavations (10). Rain is capable of distributing the conidia of F. annosus into the upper horizons of the soil column (12, 16, 19). Once in the soil, conidia may persist in a viable condition for many months (16). If during this persistence, a suitable infection court is encountered, the pathogen may gain ingress to host tissue via conidial germination and penetration. This infection process is most efficient where the host roots have been wounded (9, 17). Conidia have, however, been shown capable of initiating infection in unwounded, intact roots of loblolly pine stumps (17) and stumps of other species (10). Hüppel(14) has recently provided evidence that F. annosus conidia can infect unwounded, intact roots of axenically grown Scotch pine and Norway spruce seedlings.

The hypothesis that conidia may infect unwounded roots of intact trees under natural conditions has been discussed (11, 29) but is not supported by direct evidence. F. annosus is a specialized, root inhabiting fungus (2, 8) and its development in soil is restricted by fungistasis (cf. 3, 4, 5) and competition (6, 11, 13). Support for the hypothesis of direct infection must provide evidence to show how the fungus circumvents microbial inhibition, competition, and host defense mechanisms. We have investigated the potential of root exudates from healthy roots of mature red pine to stimulate the germination of conidia of F. annosus.

MATERIALS AND METHODS

Root exudates

Root exudates were collected during the fall 1971 from a red pine plantation planted in 1930 in south-central Connecticut. The unthinned stand had an original spacing of 2.7 m and had sustained no mortality due to F. annosus infection, although it existed in an area where several plantations have been severely influenced. In June high-wall tents (3 x 3.5 m) were erected over portions of the root systems of several trees. Woody roots, ranging in diameter from 3-13 mm, from each tree were exposed, severed and bagged as described previously (25). During September and October the bagged roots sprouted and grew vigorously. Root exudates from the unsuberized tips of 35 roots were collected (25) from five trees ranging from 21 to 29 cm diameter and 12 to 15 m height. The new woody roots were grown axenically in glass tubes for 14 days.

Conidial germination

An isolation of F. annosus was made from a sporophore developing in a nearby, infected red pine plantation. The isolate was maintained in plate culture on malt agar at 24°C. Conidia were obtained by flooding 14-day old plates and were washed by several centrifugations with sterile distilled water. Approximately 250,000 washed conidia were evenly applied to the grid surface of washed, 25 mm diameter Millipore filters (0.45 μ).

In vitro conidial germination was determined by placing filters on the surface of silica gel plates (7, 20) prepared by mixing 5 ml of potassium silicate with 5 ml of; distilled water, 10% glucose solution, 3% malt extract solution or the root exudate solution collected from a single root. Root exudate solutions were concentrated from an approximately 40 ml collection volume to 5 ml with a flash evaporator. The 5 replicate plates of each treatment were incubated at 24°C for 96 h. The filters were stained with lactophenol trypan blue and the germinated and ungerminated spores were determined in 10 randomly selected fields on each filter.

Soil was obtained from horizons of root growth at the same time root exudates were collected. The soil (pH 4.0 in 1:2 soil: 0.01M $CaCl_2$ suspension, 43% moisture content at time of collection) was slightly air dried and passed through a number 10 mesh (2 mm) sieve. Approximately 10 g (dry wt basis) was added to each of 20 porcelain crucibles. Five each of these crucibles received 2.5 ml of; distilled water, 5% glucose, 1.5% malt extract and the root exudate solution collected from a single root. Root exudate solutions were concentrated from an approximate 40 ml collection volume to 2.5 ml with a flash evaporator. Treatment solutions resulted in a final soil moisture content of about 50%. A Millipore filter with conidia was then inserted into the soil in each crucible after the method of Adams (1) and the crucibles incubated in a plastic chamber at 24°C for 96 h. The spores were stained and counted as in the silica gel plate test.

RESULTS

At the end of the 14-day collection period, only 10 root exudate samples proved free of microbial contaminants. The roots yielding these exudates ranged in dry wt from 34 to 55 mg and were from 3 trees.

The silica gel plate test indicated that *F. annosus* conidia were not stimulated to germinate by water or 5% glucose and were stimulated to germinate from 38 to 56% and 3 to 5% respectively by 1.5% malt extract and 50% red pine root exudate (Table 1).

Table 1. Germination percentages of *Fomes annosus* conidia on membrane filters on silica gel plates

Replicate filter	Distilled water	Glucose (5%)	Malt Extract (1.5%)	Red Pine root exudate (50%)
1	0.06±0.06*	0.17±0.07	54.4±2.8	3.7±0.3
2	0	0.03±0.02	55.9±3.5	4.1±0.4
3	0	0	38.1±3.2	4.4±0.5
4	0.1 ±0.09	0.16±0.08	41.9±3.8	2.8±0.6
5	0	0.09±0.06	41.7±4.0	4.8±1.0
Average	0.3	0.09	46.4	4.0

* The mean and standard error are of spore counts of 10 randomly selected fields per filter

When the Millipore filters with conidia were introduced into the soil and treated with 1.5% malt extract and 100% root exudate these materials remained stimulatory, but their effectiveness was reduced to approximately 25% of what it had been in the silica gel plates (Table 2).

Table 2. Germination percentages of Fomes annosus conidia on membrane filters buried in soil

Replicate filter	Distilled water	Glucose (5%)	Malt Extract (1.5%)	Red Pine root exudate (100%)
1	0	0	10.8±0.8*	0.9±0.1
2	0	0	8.3±2.0	1.1±0.3
3	0	0	10.1±2.1	0.8±0.2
4	0	0	11.7±1.0	1.1±0.3
5	0	0	13.3±1.8	1.3±0.5
Average	0	0	10.8	1.0

* The mean and standard error are of spore counts of 10 randomly selected fields

DISCUSSION

The autumn season is a particularly suitable time to collect root exudates for the purposes of this study as this season is also one of maximum spore production by F. annosus (10). The procedure for surface sterilization prior to axenic culture of a root for exudate collection has generally proved successful in approximately one-half the roots treated (26, 27). The fact that less than one-third of the red pine roots were successfully treated was probably due to fortuitous circumstances rather than inherent characteristics of the roots.

The maximum germination of conidia of the isolate used in this study was 56%. This is low compared with the percentage germination of conidia of other F. annosus isolates under optimum conditions (15, 21, 23). This low germination is probably not due to any peculiarities of the techniques employed in this study, for example toxicity of the membrane filters, but rather to inherent variability of this fungus (cf. 2).

The root exudates from the healthy, unsuberized root tips of new woody roots enhanced conidial germination only slightly relative to distilled water controls. When tested in unsterilized soil, this enhancement was very small (approximately 1%) but consistent for all samples tested. The greatest difficulty in extrapolating this laboratory stimulation to field stimulation under natural conditions is a failure to appreciate how much exudate is actually released per

unit of rhizosphere soil. In this experiment, we arbitrarily applied the concentrated exudate from a single root (aver. oven dry wt 42 mg) to approximately 10 g (dry wt basis) of rhizosphere soil. This amount may have been too dilute or too concentrated, depending largely on the specific sites of exudation from the root. We will be unable to improve our evaluation in this regard until we ascertain the relationships between exudation and various regions of the growing root. In all probability, however, the bulk of the exudation is from the metabolically active tip (24) and the weight of soil this tip influences is probably less than 10 g. Our test, therefore, may have been conservative and may have grossly diluted the exudate and correspondingly reduced its ability to occasion conidial germination.

The fact that conidia of F. annosus may be stimulated to germinate by red pine root exudate does not necessarily mean that infection of the root will follow. The fungus must still cope with microbial competition and antagonism of the rhizosphere (2) and successfully penetrate the intact root. Considerable evidence does exist, however, to suggest that F. annosus can compete with other soil organisms when a suitable substate is present (9, 16, 22) and that it is capable of entering healthy roots (14).

The number of woody tips potentially capable of releasing exudates stimulatory to F. annosus conidia are very numerous on a red pine tree (18) and are located in or just below the soil horizons with maximum conidial concentrations (17). It is reasonable to hypothesize, therefore, that conidia in the soil induced to germinate by root exudates may cause direct infection of intact, unwounded roots. Because of the relatively low percentage of germination induced by root exudates and the numerous constraints on F. annosus growth external to host tissue, this method of infection is probably much less important than infection via stump surfaces or wounded roots.

Acknowledgements

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MIKROSKOPISCHE ERSCHEINUNGSBILDER BEIM HOLZABBAU DURCH FOMES ANNOSUS

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EINLEITUNG

Pilzschäden können im Holz von zahlreichen Arten mit unterschiedlichem Abbauverhalten hervorgerufen werden. Für die Beurteilung pilzbefallener Hölzer ist daher die Kenntnis der Fäuleursache oftmals eine wichtige Voraussetzung. Als Hilfsmittel für die Pilzbestimmung können u.a. auch mikroskopische Untersuchungsmethoden herangezogen werden. Sie erlauben nicht nur genauere Einblicke in die Vorgänge beim Holzabbau durch Pilze sondern vermögen mitunter - infolge unterschiedlicher Intensität und Eindringtiefe der ligninabbauenden Enzyme sowie charakteristischer Abbaumuster mancher Pilze - wichtige Hinweise auf die Identität eines Holzzerstörers zu geben. Im folgenden soll versucht werden, für Fomes annosus, den wichtigsten Erreger der verbreiteten Stammfäulen in Fichten- und Kiefernbeständen, den Ablauf des Pilzangriffs im Holz zu verfolgen und einige charakteristische mikroskopische Merkmale für diese Fäuleart darzustellen.

METHODISCHES

Nach unseren Erfahrungen haben sich zur Untersuchung von Nadelhölzern, die von Weißfäulepilzen, wie z.B. den häufigsten Erregern von Wundfäulen und Lagerschäden - Stereum sanguinolentum und Stereum areolatum -

oder den bekannten Rotfäulepilzen Fomes annosus, Armillaria mellea und Odontia bicolor angegriffen sind, zwei mikroskopische Färbeverfahren gut bewährt (5, 6).

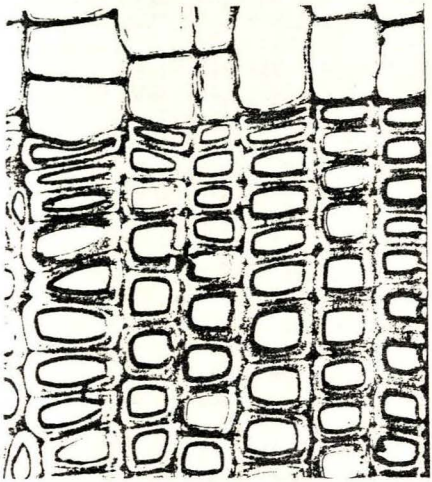
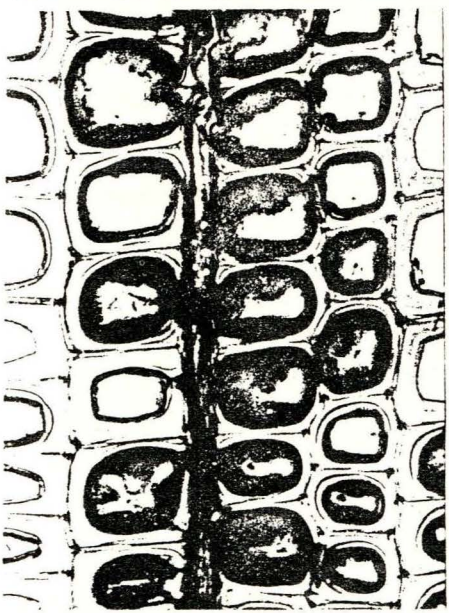
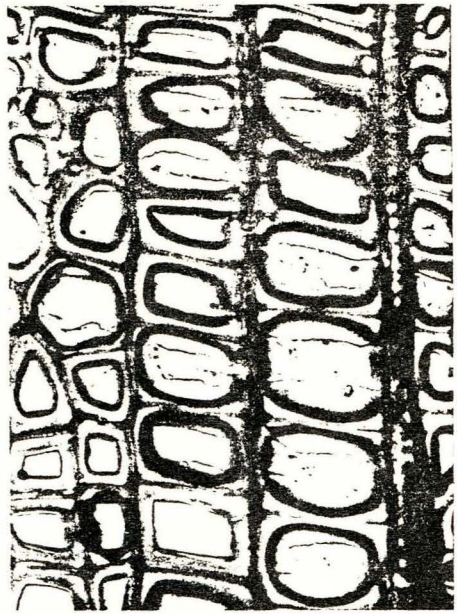


Abb. 1 Durch Fluorochromierung mit Acridin-Orange sichtbar gemachte enzymatische Einflußbereiche im frühen Angriffsstadium (Abbaustadium I)
Vergrößerung 35o : 1



a



b

Abb. 2a Durch Färbung mit Safranin und Astrablau sichtbar gemachte entlignifizierte Zellwandschichten (Abbaustadium II)
Vergrößerung 56o : 1

Abb. 2b Abbaustadium wie in Abb. 2a: Bei Fluorochromierung mit Acridin-Orange bleiben die entlignifizierten Zellwandschichten (Abbaustadium II) ungefärbt, während in den anschließenden Wandbereichen das Abbaustadium I durch Farbanreicherung dunkel gekennzeichnet ist. Vergrößerung 56o : 1

Erste Anzeichen eines Pilzangriffs lassen sich nach Fluorochromierung der Holzdünnschnitte mit Acridinorange im Fluoreszenzmikroskop erkennen, wobei auch Spuren der Enzymeinwirkung durch einen deutlichen Farbumschlag gut sichtbar werden (Abbaustadium I, Abb. 1). Auch feinste Bohrlöcher, die oft schon nach einer Einwirkungszeit von nur wenigen Tagen erscheinen, im normalen Lichtmikroskop aber noch nicht erkennbar sind, heben sich durch ihre dunkle Umrandung gut von der übrigen Zellwand ab. Diesem Farbumschlag entspricht eine - im allgemeinen nicht sehr deutliche - Farbanreicherung bei der Färbung mit Safranin. Bei fortgeschrittenem Holzabbau, wenn bereits ein gewisser Ligninabbau in den Zellwänden stattgefunden hat (Abbaustadium II), empfiehlt sich die Doppelfärbung mit Safranin und Astrablau, da die weitgehend entlignifizierten Stellen sich kräftig mit Astrablau anfärben (Abb.2a) während sie das Fluorochrom Acridinorange nicht mehr aufzunehmen vermögen (Abb. 2b). Auch für die Darstellung von Pilzmycel ist dieses Verfahren, ähnlich wie die 1929 von CARTWRIGHT vorgeschlagene Färbung mit Safranin und Pikrinanilinblau, gut geeignet. Darüberhinaus erlauben alle diese Färbungen auch eine Beurteilung der Vitalität bzw. des Alterszustandes der Hyphen (7). Für die vorliegenden Untersuchungen wurde Mikroschnitte von kleinen frischen Fichtenholzproben entnommen, die zuvor eine gewisse Zeitlang dem Angriff von Fomes annosus ausgesetzt und sodann in 7o%igem Alkohol aufbewahrt waren. Die Versuchszeiten waren gestaffelt und betrugen in der Regel 1o, 3o, 9o und 15o Tage. In einigen Fällen wurden von den selben Proben Präparate für ergänzende Untersuchungen am Rasterelektronenmikroskop gewonnen, wodurch die lichtmikroskopischen Ergebnisse vielfach noch vertieft werden konnten.

Zur Beurteilung der hier wiedergegebenen Ergebnisse muß bemerkt werden, daß sie zwar eine gute Vorstellung des Verhaltens von Fomes annosus im Holz zu geben vermögen, daß sie aber in ihrem zeitlichen Ablauf nicht festgelegt werden können. Wenn eine Pilzinfektion zustandegekommen ist und sich bereits etwas im Holz ausgebreitet hat, sind in den befallenen Holzproben fast immer frühe und fortgeschrittene Abbaustadien nebeneinander zu finden. Für den Fortgang der Holzzersetzung ist nicht nur die Holzfeuchtigkeit und die derzeitige Virulenz des angreifenden Pilzstammes entscheidend, sondern er wird außerdem auch von einer Reihe oft nicht erfaßbarer Faktoren und Zufälligkeiten beeinflußt. Aus diesem Grund variieren die Ergebnisse solcher Untersuchungen nicht nur bei Verwendung verschiedener Teststämme sondern unterliegen auch innerhalb der gleichen Versuchsserie oder bei Wiederholungen unter gleichen Versuchsbedingungen oftmals erheblichen Schwankungen. Trotzdem lassen sich aus dem sich stets wiederholenden Auftreten typischer Abbauerscheinungen und -stadien gewisse Gesetzmäßigkeiten ableiten, die für die Abbautätigkeit bestimmter Holzzerstörer kennzeichnend sind. Die Beobachtungen, über die hier berichtet wird, wurden mit einem gutwüchsigen und verhältnismäßig intensiv abbauenden Pilzstamm gewonnen, der jedoch bei einer Wiederholung der Tests zu einem späteren Zeitpunkt seine Virulenz in ungewöhnlichem Maße gesteigert hatte. Die folgenden Zerstörungsbilder wurden im allgemeinen in beiden Versuchsserien festgestellt, auf Ausnahmen wird eigens hingewiesen.

HOLZABBAU DURCH FOMES ANNOSUS

Abbauerscheinungen im Holz

Die erste Überprüfung des Abbauverhaltens von Fomes annosus im Holz fand nach 1otägigem Angriff statt. Zu diesem Zeitpunkt waren im Fluoreszenzmikroskop bereits Spuren der Pilzeinwirkung sichtbar, in den Markstrahlen und in einigen Spätholztracheiden konnte stellenweise ein deutlicher Farbumschlag beobachtet werden (Abb. 1). Intensität und Ausbreitungsgeschwindigkeit der Enzyme sind - wie schon früher beobachtet (2, 4) - relativ gering, es wird zunächst nur eine schmale Zone um das Zellumen bzw. in der unmittelbaren Umgebung der Hyphen davon erfaßt. Außerdem können bereits einzelne winzige Bohrlöcher vorhanden sein. Nach 3o Tagen hatte sich noch nicht viel verändert, lediglich die enzymbeeinflußten, durch Farbumschlag gekennzeichneten Bereiche hatten sich, vor allem innerhalb des parenchymatischen Markstrahlgewebes, etwas vergrößert und es fanden sich neben den winzigen Bohrlöchern auch schon vereinzelt etwas größere. Nach 3monatiger Angriffszeit war dann gelegentlich in Spätholztracheiden das fortgeschrittene Abbaustadium zu erkennen, in dem die dem Zellumen am nächsten liegenden Wandschichten schon weitgehend entlignifiziert sind und das Fluorochrom nicht mehr aufnehmen sondern im Fluoreszenzmikroskop als amorphe gequollene Substanz erscheinen. Dieses Stadium läßt sich - wie schon erwähnt - durch die Doppelfärbung des Schnitts mit Safranin und Astrablau gut darstellen (Abb. 2a). Dieser Zustand ist offenbar nur ein kurzes Übergangsstadium, dem rasch die vollständige Auflösung der betreffenden Zellwandschichten folgt. Tatsächlich waren zum gleichen Zeitpunkt auch bereits erste Auflösungserscheinungen in der Zellwand zu beobachten, die bevorzugt die Tori der Hoftüpfel erfaßten, aber auch an anderen Stellen der Spätholztracheiden festgestellt wurden (Abb. 3).

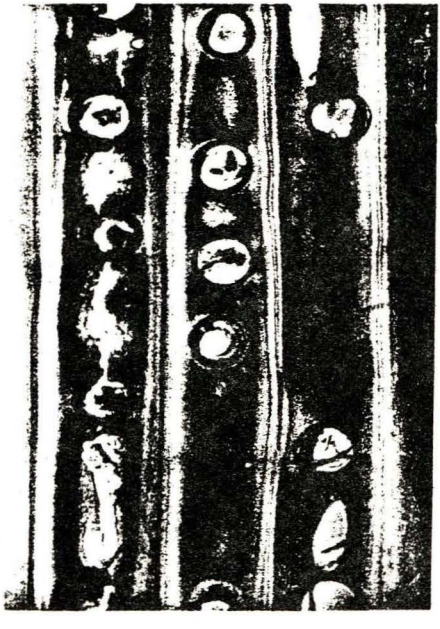


Abb. 3 Wandauflösungen in den Fichtentracheiden nach 9otägigem Befall durch Fomes annosus. Fluorochromierung mit Acridinorange
Vergrößerung 56o : 1

Auch entlang der Hyphen, die vielfach nur eine relativ kurze Lebensdauer zu haben scheinen undin den Probeklötzchen nach 3monatiger Einwirkungszeit zum großen Teil bereits abgestorben waren, konnten gleichzeitig deutliche Enzymeinflußbereiche beobachtet werden. Ähnliche Lysiszonen wurden von PEEK (8) schon nach etwa 3 Wochen nachgewiesen. Nach 5 Monaten war die fluoreszenzoptisch erkennbare, vom Zellumen ausgehende, kontrastreiche braune Pilzeinwirkungszone nach wie vor schmal, das gequollene, amorphe Zwischenstadium war nun aber häufiger zu finden und die Zellwände waren an zahlreichen Stellen in größeren Bereichen teilweise oder ganz aufgelöst. Auffallend waren in diesem Stadium, zumindest in den Probeklötzchen, die dem Pilz in seiner besonders virulenten Phase ausgesetzt waren, zahlreiche, in allen Richtungen in der Zellwand verlaufende Wanddurchbohrungen, wodurch im Querschnitt des angegriffenen Holzes ein Fäulebild entstand, das an Moderfäulebefall erinnert (Abb. 4). Im Gegensatz zu diesem Fäuletyp war aber im safranin astrablau-gefärbten Schnitt um die entstandenen Bohrlöcher die gleiche dunkelrote Hofbildung aufgefallen wie um das Zellumen, was eine für die Anfangsphase von Weißfäulebefall typische Erscheinung ist. Im Längsschnitt waren entlang der Hyphen ebenfalls deutliche Lysiszonen zu beobachten, jedoch nicht die für Moderfäulen charakteristischen Kavernen. Auf Grund dieser Merkmale ist die Fomes-Fäule auch bei beträchtlicher Abbauintensität des Pilzes, die durch eine extreme Bohrlochbildung gekennzeichnet sein kann, eindeutig von Moderfäulebefall zu unterscheiden.

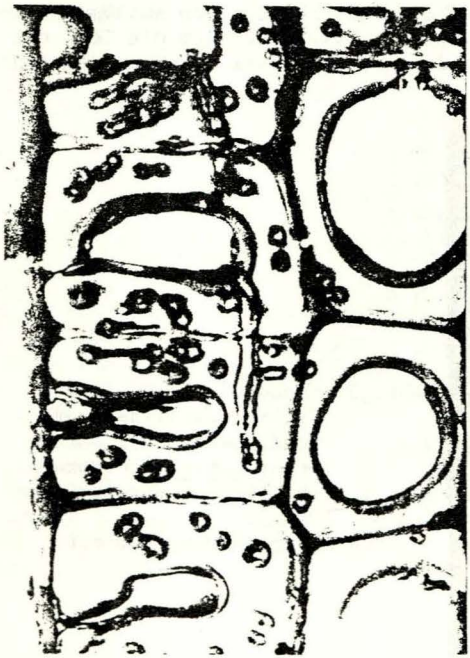
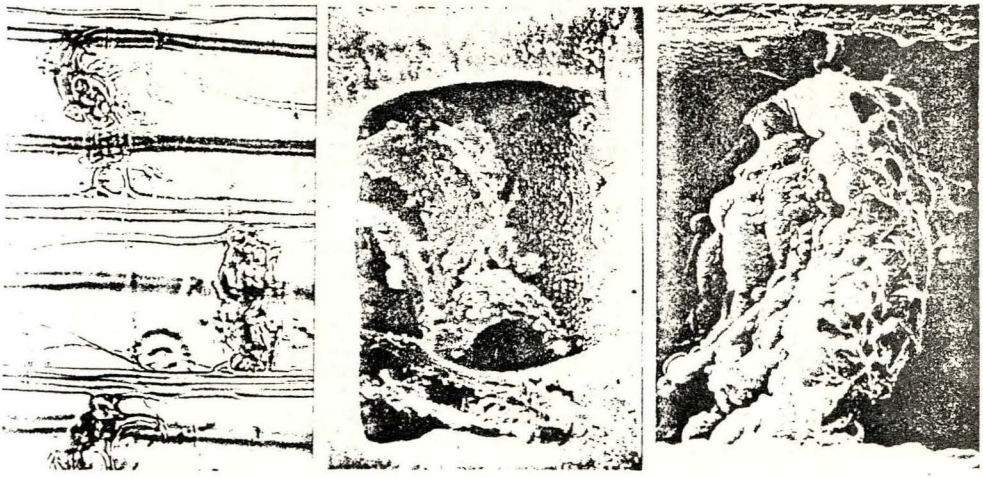


Abb. 4 Extreme Häufung von Bohrlöchern nach Einwirkung eines besonders virulenten Fomes annosus-Stammes. Färbung mit Safranin/ Astrablau. Die dunklen Zonen erscheinen tiefrot, was auf das Abbaustadium I hinweist. Vergrößerung 1400 : 1

Pseudoparenchymatische Mycelanhäufungen

Ein besonders auffälliges Merkmal der Pilztätigkeit im Holz stellen die von verschiedenen Weißfäuleerregern gebildeten pfropfenartigen Zwischenwände aus pseudoparenchymatischen Mycelanhäufungen in den Tracheiden dar, die oftmals die deutlich pilzbeeinflußten Bereiche gegenüber noch pilzfreien Stellen abgrenzen. Solche von CAMPBELL (5) als "Pseudosklerotien" (das sind Organe, die unter ungünstigen Wachstumsbedingungen entstehen und dem Pilz eine spätere vegetative Vermehrung ermöglichen) bezeichnete Mycelanhäufungen werden zwar von Fomes annosus nicht so häufig angelegt wie beispielsweise von Armillaria mellea oder Trametes pini (2), doch waren sie in den untersuchten Präparaten nach 15o-tägiger Versuchszeit wiederholt angetroffen worden (Abb. 5b und c). Ihre Entstehung veranschaulicht Abb. 5a. An den Hyphen bilden sich zunächst zahlreiche keulenförmig verdickte Auswüchse, die Pilzfäden lagern sich eng zusammen und verflechten sich in den Tracheiden auf engem Raum zu kompakten Zwischenwänden mit rauhen, granulierten Oberflächen. An Hand ihrer Oberflächenbeschaffenheit lassen sie sich bei starken Vergrößerungen (z.B. im Rasterelektronenmikroskop) von vergleichbaren Mycelbildungen anderer Pilze unterscheiden.



a b c

Abb. 5 Pseudoparenchymatische Mycelanhäufungen im Lumen von Fichtentracheiden
- a im Lichtmikroskop, ungefärbtes Präparat, Vergrößerung
- b Rasterelektronenmikroskopische Aufnahme Vergrößerung 182o : 1
- c Rasterelektronenmikroskopische Aufnahme Vergrößerung 2loo : 1

Hautartige Bildungen und Einschlüsse im Zellumen

Außer diesen beim Angriff von Weißfäulepilzen im Nadelholz in ähnlicher Form häufiger vorkommenden Pseudosklerotien sind bei Fomes annosus-Befall in den Faserzellen immer wieder hautige Gebilde zu beobachten, deren Auftreten oft deutlich mit dem Pilzmycel in Zusammenhang steht, deren Funktion aber noch nicht geklärt ist. Oft sind bereits nach einer 1otägigen Einwirkungszeit die in Abb. 6a dargestellten dünnen Häutchen zu finden, die im Längsschnitt mitunter die gesamten Tracheiden auskleiden (Abb. 7a). Interessanterweise treten sie bevorzugt in solchen Holzfasern auf, in denen sonst noch keine Spuren der Pilztätigkeit erkennbar sind (Abb. 6c). Mit fortschreitender Einwirkungsdauer scheinen sich die Häutchen deutlich zu verdicken, denn nach 3otägigem Angriff sind im Zellinneren dann vielfach kompakte Häute anzutreffen, deren Oberfläche - wie Abb. 6b zeigt - eine grobkörnige Struktur besitzt.

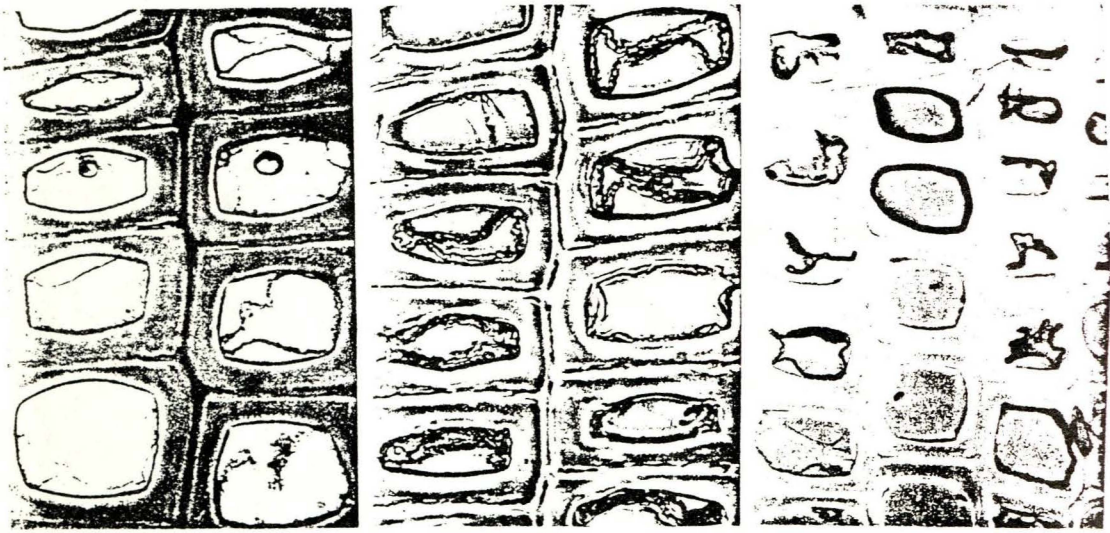


Abb. 6 Hautartige Bildungen in den Lumina von Fichtentracheiden

a nach 1otägigem Befall durch Fomes annosus
Färbung nach CARTWRIGHT,
Vergrößerung 88o : 1

b nach 3otägigem Befall durch Fomes annosus
Ungefärbtes Präparat
Vergrößerung 88o : 1

c nach 3otägigem Befall durch Fomes annosus. Acridin-Orange.
Vergrößerung 56o : 1

Die Vermutung, es könne sich bei diesen Hautbildungen um angegriffene Schichten der Zellwand handeln, die sich bei der Präparation abgelöst haben, dürfte - abgesehen von der Tatsache ihres Auftretens in noch nicht angegriffenen Tracheiden - durch den Hinweis zu widerlegen sein, daß nach der kurzen Angriffszeit von nur 1o Tagen im Holz lediglich Spuren einer enzymatischen Beeinflussung, aber noch keinerlei Abbauerscheinungen zu erkennen waren und es daher wenig wahrscheinlich ist, daß sich zu diesem Zeitpunkt bereits einzelne Schichten von der Zellwand abspalten lassen. Außerdem müßten in einem solchen Fall ähnliche Bilder gelegentlich auch durch die Einwirkung anderer Pilze, insbesondere solcher, die die Holzsubstanz rascher angreifen als Fomes annosus, zustandekommen. Wir haben diese Gebilde bisher jedoch nur als Folge der Einwirkung des Wurzelschwamms festgestellt, so daß die Hautbildungen sogar als charakteristisches Merkmal für Fomes annosus-Befall gewertet werden können.

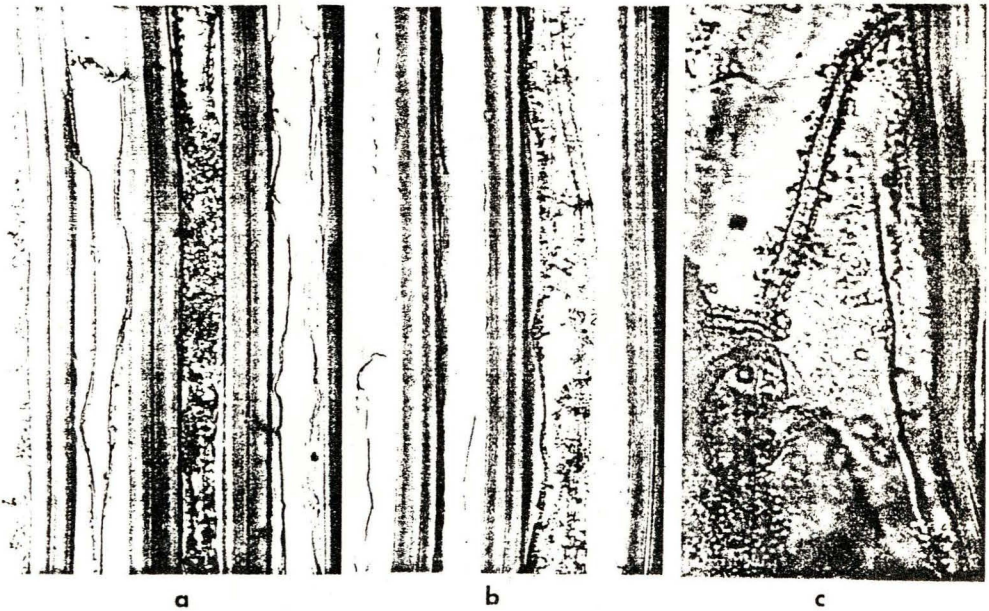
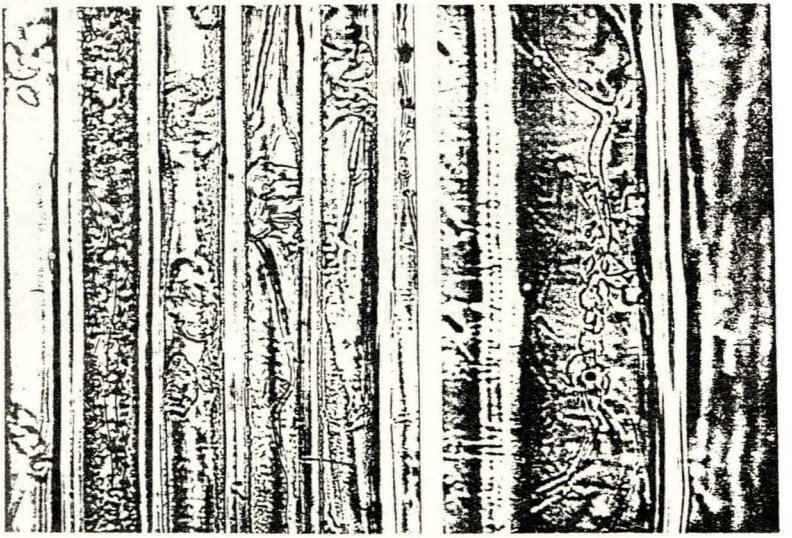


Abb. 7 Hautartige Bildungen im Lumen von Fichtentracheiden im Zusammenhang mit körnigen Einschlüssen und Mycel nach 1otägiger Befallsdauer.
Vergrößerung:
a = 56o : 1
b = 88o : 1
c = 14oo: 1

Oftmals sind aber in den Tracheidenhohlräumen nicht nur hautartige Bildungen zu finden, sondern sie sind mehr oder weniger mit Einschlüssen ausgefüllt, die in ihrer Beschaffenheit mitunter an die dickeren Häute erinnern, aber auch amorphen, feinkörnigen oder kristallinen Charakter haben können. Ähnlich wie die feinen Häutchen sind auch solche Einschlüsse bereits nach 1otägigem Befall zu finden und wurden wiederholt gerade entlang des Pilzmycels beobachtet. Über ihre Entstehung lassen sich ebenfalls nur Vermutungen äußern. Da sie schon im frühesten Angriffsstadium auftreten, ist es unwahrscheinlich, daß diese Zellinhaltsstoffe durch den Zerfall der beschriebenen Häute oder des Pilzgewebes zustandekommen, es erscheint eher denkbar, daß sie sich aus gewissen Stoffwechselprodukten bilden, wie möglicherweise die beschriebenen Häute auch. Eine eindeutige Unterscheidung zwischen Häuten und sonstigen Zellinhaltsstoffen ist nicht immer möglich, da beide Erscheinungen oftmals auf engstem Raum zusammen vorkommen und sich dann kaum gegeneinander abgrenzen lassen. Manchmal entsteht der Eindruck, daß sich granulierte Einschlüsse zu Häuten verbinden können. In Abb. 7b und c sind solche Bildungen zu sehen, die offensichtlich mit dem Mycel in Zusammenhang stehen.

Ebenfalls im Zusammenhang mit der Ausbreitung von Fomes annosus im Holz steht die auffallende Anreicherung von kristallinen Einschlüssen in den Tracheidenlumina der sogenannten Reaktionszone, die von lebenden Bäumen als Übergangszone zwischen gesundem Splint- und faulem Reifholz gebildet wird, wenn die Fäule bereits den gesamten Kern erfaßt hat und im Splintbereich vordringt (1).



a b

Abb. 8 Verschiedene kristalline Einschlüsse im Lumen von Fichtentracheiden. Ungefärbte Präparate.
Vergrößerung:
a = 56o : 1
b = 88o : 1

Abb. 8a zeigt reichliche Kristallbildung in den von Pilzmycel durchzogenen Tracheiden aus einer bereits braun verfärbten Zone, in Abb. 9 ist daneben die anschließende sogenannte grüne Zone mit zahlreichen kleineren Kristallen abgebildet, in der ebenfalls noch Hyphen zu finden sind. Auffallend ist, daß diese kleineren Formen die vielfach zusammen mit körnigen Einschlüssen auftreten, oft gerade an den Hyphen angelagert oder entlang der Spuren bereits abgestorbenen Mycels zu finden sind (Abb. 8b). Hinsichtlich ihrer Form und Größe sind die Kristalle sehr vielgestaltig, es treten sowohl kubische, hexagonale oder rhombische wie auch nadel- oder sternförmige Körper auf. Auch in ihrer chemischen Zusammensetzung zeigt sich keine Übereinstimmung, vielmehr verhalten sich die einzelnen Kristalltypen gegenüber verschiedenen Lösungsmitteln recht unterschiedlich, doch sind sie alle mehr oder weniger rasch in verdünnter Salz- oder Schwefelsäure löslich.

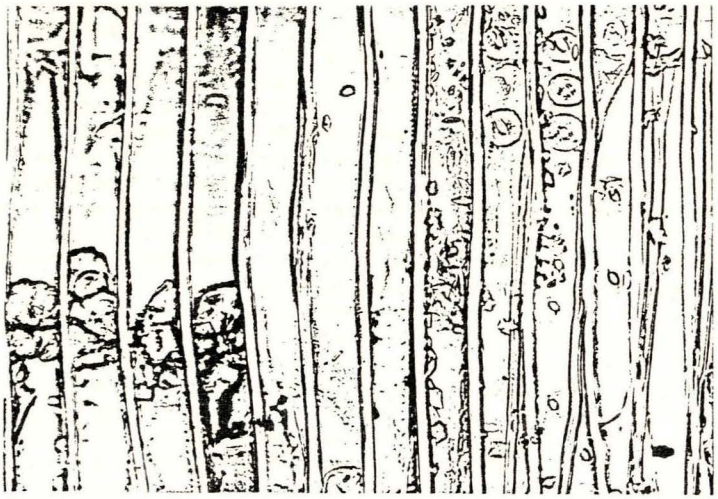


Abb. 9 **Verschiedene kristalline Einschlüsse im Lumen von Fichtentracheiden.**
Links: von Fomes annosus braun verfärbte Zone
Rechts: Reaktionszone (sog.grüne Zone)

Vergrößerung: 35o : 1

ZUSAMMENFASSUNG

Zusammenfassend läßt sich feststellen, daß der Ablauf des Holzabbaus durch Fomes annosus im wesentlichen mit dem Verhalten einer Reihe schon früher getesteter Weißfäulepilze übereinstimmt (4, 9). Der Enzymeinfluß, der schon wenige Tage nach Beginn der Pilzausbreitung durch eine Veränderung der Sekundärfluoreszenz im Holz erkennbar ist, beschränkt sich zunächst auf die unmittelbare Umgebung der Hyphen und macht sich bevorzugt in den Holzstrahlen bemerkbar. Die vom Lumen aus in die benachbarten Zellwandschichten diffundierenden Enzyme erreichen, verglichen mit denen anderer Fäuleerreger wie u.a. beispielsweise Stereum sanguinolentum oder Stereum areolatum, eine geringere Eindringgeschwindigkeit und -tiefe. Auch später bleibt der durch Acridinorange sichtbar werdende enzymatisch beeinflußte Bereich relativ schmal. Dafür setzt schon bald das folgende Abbaustadium ein, in dem die angegriffenen Stellen schon stark entlignifiziert sind und, da sie das Fluorochrom Acridinorange nicht aufnehmen, im Fluoreszenzmikroskop ungefärbt, gequollen und amorph erscheinen, sich aber andererseits mit dem Cellulosereagens Astrablau kräftig anfärben. Dieses Stadium, das fast alle Weißfäuleerreger im Nadelholz verursachen, wird rasch durchlaufen und es folgt bald die völlige Auflösung der beeinflußten Zellwandschichten. Die Durchbohrung der Zellwände durch die Pilzhyphen geht ähnlich vor sich wie bei anderen Fäuleerregern. Von der Hyphenspitze werden ligno- und cellulolytische Ektoenzyme abgeschieden, die die Zellwand an dieser lokal eng begrenzten Stelle auflösen, so daß die am Hyphenende gebildete Mikrohyphe in die Zellwand einzudringen vermag (8). Unter der Einwirkung eines zur Zeit der Versuchsdurchführung besonders virulenten, intensiv angreifenden Fomes annosus-Stammes fand eine ungewöhnlich starke, in allen Richtungen die Zellwand durchdringende Bohrlochbildung statt, die in ähnlicher Form noch von keinem anderen Holzzerstörer verursacht wurde. Querschnitte der befallenen Fichtenholzproben zeigten eine moderfäuleähnliche löcherige Zerstörung der Sekundärwand, wobei jedoch in der enzymbeeinflußten Zone um diese Bohrlöcher wie um das Zellumen bei der Färbung mit Safranin und Astrablau durch Anreicherung des roten Farbstoffs eine deutliche Hofbildung hervorgerufen wurde. In diesem Zusammenhang sei darauf hingewiesen, daß die Abbautätigkeit von Fomes annosus im Holz wie bei fast allen Holzzerstörern hinsichtlich ihrer Intensität beträchtliche Schwankungen aufweisen kann. Hierbei ist nicht nur die unterschiedliche Virulenz verschiedener Stämme des gleichen Pilzes von Bedeutung, auch der selbe Stamm verändert vielfach von Zeit zu Zeit seine Aggressivität. Welche Faktoren dafür verantwortlich sind, ist bisher im einzelnen nicht bekannt.

Übereinstimmend mit vielen anderen Weißfäuleerregern bewirkt auch Fomes annosus die dunklen Zonenlinien, die oftmals pilzbefallene gegenüber nicht angegriffenen Stellen im Holz abgrenzen und durch pseudoparenchymatische Mycelanhäufungen, die als pfropfenartige Zwischenwände die Tracheidenlumina verstopfen, zustandekommen. Die von Fomes annosus gebildeten Mycelpfropfen unterscheiden sich von ähnlichen Mycelbildungen anderer Pilze durch ihre rauhe, stark granulierte Oberflächenbeschaffenheit.

Ein Charakteristikum für die Abbautätigkeit von Fomes annosus, das unter der Einwirkung anderer Holzzerstörer bisher niemals beobachtet wurde, ist die Bildung von zunächst feinen, später kompakteren Häuten in den Zellumina. Sie treten schon zu einem Zeitpunkt auf, wo außer geringen Spuren eines Enzymeinflusses noch keinerlei Hinweise auf die Pilztätig-

keit zu finden sind. Es dürfte sich daher wohl kaum, wie zunächst vielleicht angenommen werden könnte, um eine durch die Präparation verursachte Ablösung bereits angegriffener Wandschichten handeln, zumal sie meist in noch nicht durch die Enzymeinwirkung veränderten Tracheiden auftreten. Es wäre jedoch denkbar, daß für ihre Bildung gewisse Stoffwechselprodukte des Pilzes eine Rolle spielen. Mit Sicherheit dürfte sie aber mit dem Pilzmycel im Zusammenhang stehen. In reichlichem Ausmaß treten außerdem bei Fomes annosus-Befall mehr oder weniger feine körnige Einschlüsse im Zellinneren auf, wie sie manchmal in ähnlicher Form auch beim Angriff anderer Pilze, insbesondere im Eichenholz von Fistulina hepatica bewirkt werden (3). Häufig nehmen die Zellinhaltsstoffe auch kristallinen Charakter an, solche Kristalle sind vornehmlich in der sogenannten Reaktionszone zwischen befallenem Kern- und gesundem Splintholz zu finden. Form und chemische Zusammensetzung der Zelleinschlüsse variieren erheblich und über ihre Entstehung und Funktion ist noch keine klare Aussage möglich. Weitere chemische Untersuchungen erscheinen daher wünschenswert.

SUMMARY

Slides of small Norway spruce specimens attacked by the red rot fungus Fomes annosus were studied by microscopical methods to derive knowledge from the process of decomposition of the cellwalls and to observe some characteristic features of this type of wood decay. For staining the slides two preparation methods were used: contrast staining with Safranin and Astrablue and fluorescence-microscopical tests with the fluorochromatic dye Acridin-orange. The results were completed by investigations with the scanning electron microscope.

The decomposition of the cellwalls by Fomes annosus generally proceeds rather slowly, the progress corresponds with that of other white rot fungi. Sometimes, when the attacking strain was extremely virulent, a great number of bore holes were found in the secondary wall. Fomes annosus also produces pseudoparenchymatous accumulations of mycelium with rough granular surfaces, which cork the fibres. Skin-like formations and a great deal of amorphous, grained or cristalline inclusions whose function is still unknown, appeared in the luminaries of the attacked tracheids.

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FOMES ANNOSUS (FR.) COOKE, PENIOPHORA GIGANTEA (FR.) MASSEE, AND OTHER FUNGI COLONISING STUMPS OF PI - NUS SYLVESTRIS L.

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INTRODUCTION

Pine stumps established in a tree stand, if not trea - ted specially, may be subject of infection by Fomes annosus, or they may, because of different circumstances (lack of spores of the pathogen in the air, inconvenient meteorolo - gical conditions, a.s.o.), escape from this threat. Their vicissitudes seem to be dependent most on the site and on some weather factors occuring within it. However, the en - vironmental influences may be more or less precisely ex - pressed in terms of the microflora inhabiting the surfice or the inner parts of the host plant. Hence, it may be of interest to pay attention to microorganisms other than pa - thogenic ones, penetrating tissues of the host.

The goal of this paper is to present some results ob - tained in the course of investigations on Fomes annosus and Peniophora gigantea as inhabitants of pine stumps, and on the relation of other stump fungi to the two mentioned ones.

I. DYNAMICS OF STUMP COLONISATION BY FUNGI

In the year 1967 have been choosen two Scots pine stands, one of them, about 45 years old, in the Forest Sieraków (section 73), the other one, about 30 years old, in the Forest Klęka (section 107). The latter one was a stand of first rotation, established on abandoned farm land, of rather good site conditions, and strongly affected by butt rot. The stand in Forest Sieraków has been established after the pine stand existing earlier was destroyed by a Panolis flammea calamity. The stand considered now, was at the time of investigation destroyed to a great extent again, in this case by Fomes annosus. Its site was a poor one (precipitation about 500 mm per year, a relatively shallow sandy soil with Cladonia spp. occuring on its surfice, the hight of the stand amounting about 8 m).

Each week (beginning in April, ending in October) 4 healthy stumps have been established (on the whole 115 stumps in the Forest Klęka, and 112 ones in the Forest Sieraków). In the next Autumn samples in form of discs have been taken from those stumps to isolate fungi from them. From each sample were taken 48 particles of wood (inocula), and plated on the agar of Kuhlman and Hendrix (1962).

One part of results obtained is yet published (Mańka, Przezbórski and Pukacki, 1972), therefore only a condensed information about these results is given here. From 5520 were obtained 376 isolates of Fomes annosus, 2361 of Peniophora gigantea, and over 2500 isolates of other fungi. From table 1 is evident that : 1. No Fomes annosus infections

Table 1. - Numbers of isolates (in % % of inocula) of Fomes annosus and of Peniophora gigantea obtained from stumps established at different terms

Terms of stump establishment	Fomes annosus	Peniophora gigantea
8. IV 67	0	23
15 IV 67	0	21
22 IV 67	0	67
29 IV 67	0	3
6 V 67	0	70
13 V 67	0	40
20 V 67	5	44
27 V 67	4	31
3 VI 67	0	80
10 VI 67	19	32

17	VI 67	0	18
24	VI 67	0	69
1	VII 67	0	57
8	VII 67	0	65
15	VII 67	21	38
21	VII 67	33	5
29	VII 67	12	61
5	VIII 67	15	37
12	VIII 67	0	81
19	VIII 67	0	10
26	VIII 67	16	41
2	IX 67	4	35
16	IX 67	5	46
23	IX 67	22	22
30	IX 67	10	61
7	X 67	18	39
14	X 67	5	55
21	X 67	15	68

were detected in stumps established in April and in May , very few in those of June and July, many in stumps estab - lished later. 2. Peniophora gigantea was isolated from all stumps, in most cases abundantly.

In the investigations accomplished in the Forest Sieraków (results not yet published) participated also Dr.S. Ko - walski. From 5376 inocula plated in this case have been obtained numerous isolates of Peniophora gigantea (from April- and May-stumps even as much as about 80 % of all fungal isolates), whereas Fomes annosus isolates not at all ! Other fungi as the two ones mentioned were isolated more abundantly from stumps established in later months. The lack of Fomes annosus isolates was a little astoni - shing, therefore about 30 pine root systems have been ex- cavated and fungi were from them isolated to elucidate questions which emerged unexpectedly. From nearly all of those root systems Fomes annosus was isolated easily, in some cases also Armillariella mellea, frequently "other" fungi (Mycelium radicis atrovirens, Trichoderma spp., a.s.o.), however no isolates of Peniophora gigantea have been obtained (probably owing to the fact that only root systems of still standing trees have been excavated and analysed). The infection of the roots investigated by Fo- mes annosus could occur secondarily. The lack of Fomes annosus in the stump wood came probably as well from cli- matical as from soil conditions, which favoured an early drying up of the stumps, and so created unfavourable cir- cumstances for infection of the latter ones by the patho- gen mentioned.

II. PENETRATION OF PINE STUMPS BY FOMES ANNOSUS AND OTHER FUNGI DEPENDENTLY ON THE BREADTH OF THE ANNUAL INCREMENT RINGS

In Autumn of the year 1972, the senior Author with some participants (Wanda Dyspolska, Małgorzata Mańka, and L. Szurkowski), choosed two Scots pine stands, a 30 years old one in the Forest Zielonka (section 38 f) known as nearly free of Fomes annosus losses, and a 25 years old one in the Forest Klęka (section 41 g)known to be strongly attacked by that pathogen. In each of those stands 12 stumps have been established. 6 stumps of each group evidenced a small diameter (in Zielonka 7.0-8.5 cm; in Klęka 5.0-7.5 cm), and 6 ones a great diameter (in Zielonka 12.5-15.0 cm; in Klęka 12.0 cm and more). After 2 months of exposure samples of the stumps in form of discs were taken to isolate fungi from them. From each disc 180 inocula were plated on a 4 % malt agar. The results are given in table 2.

Table 2. - Number of isolates of Fomes annosus, Peniophora gigantea, and other fungi, obtained from stumps

Forest	Stumps	Numbers of isolates			Breadth of the annual increm. ring (mean) mm
		Fomes annosus	Peniophora gigantea	Other fungi	
Zielonka	thin	4	113	444	1.4
"	thick	3	1288	527	2.3
Klęka	thin	22	46	191	1.5
"	thick	44	1472	684	3.3

In table 2 were taken into account coefficients for making comparable numbers of isolates obtained from thin stumps (equal numbers of inocula were plated from thin as well as from thick stumps) with those from thick ones. It is evident from it, that stumps with broad annual increment rings were much more penetrated (in principle) by Fomes annosus and other fungi, and that Fomes annosus were much stronger represented in stumps from the Forest Klęka than in stumps from the Forest Zielonka, what seems to be in accordance

with the greater incidence of butt rot in the Forest Klęka known from the praxis. Moreover it should be informed, that the quantitative ratio of the Fomes annosus isolates from the Forest Klęka to the isolates of this fungus from the Forest Zielonka amounted for thin stumps 5 : 1, and for thick ones 15 : 1, whereas the ratio of Peniophora isolates from those two Forests was for thin stumps 1 : 5, and for thick ones 1 : 1. This agrees also with the known prevalence of Fomes annosus destructive activity in the Forest Klęka.

III. COMMUNITIES OF STUMP FUNGI AS CRITERION FOR ESTIMATING THE ENVIRONMENTAL INFLUENCE ON FOMES ANNOSUS

The junior Author investigated (consulting the senior Author) some differently aged Scots pine stands localised in the Forests Zielonka and Laski. The pine stands in the Forest Zielonka are known to be nearly free of Fomes annosus attack, whereas those in the Forest Laski suffer from such an attack seriously. In each of the investigated stands were cut 12 healthy trees (6 from them in the Spring, the others in the Autumn), and the stumps established thereby exposed for a time (1, 6, 12, 18, 24, and 30 months). Then discs were taken from the stumps (in some cases twice or three times at different terms) to isolate fungi from them. Fungi isolated from particular groups of stumps have been considered to be communities. It should be added, that 60 inocula were plated from each stump-disc. At last the effect of particular fungal communities on Fomes annosus as well as on Peniophora gigantea was investigated according to the biotic series method of Mańka (1965, 1970).

On the whole, from 32 666 inocula have been obtained 23 234 isolates of fungi. Fomes annosus and Peniophora gigantea were isolated more frequently from sap wood as from hard wood, the latter of the fungi more numerously from stumps established in the Spring, the former one more often from Autumn stumps, both more numerously from the upper part (depth about 1 cm) than from the deeper part (about 5 cm) of the stumps. When discs were taken the second time (6 months after taking the first disc), Fomes annosus was isolated less and Peniophora gigantea more frequently than from the discs taken earlier. A similar effect was observed in an other work (Mańka and Przezbórski, 1972), namely as a consequence of stump-debarking executed immediately after the stumps have been established. No infection of the debarked stumps occured in that case, whereas Peniophora was isolated from them more abundantly as from undebarked stumps, which moreover were evidently attacked by the pathogen lacking in debarked stumps.

Tables 3 and 4 are given here as examples only of the mode of studying the effect of defined fungal communities on particular organisms (in this case on Fomes annosus and on Peniophora gigantea), applied in this work.

Table 3 - The effect of the community of stump fungi from a 30 years old pine stand (Forest Zielonka, section 39 d) on Fomes annosus and on Peniophora gigantea

Species of fungus	Frequency of occurence	Fomes annosus		Peniophora gigantea	
		biotic effect		biotic effect	
		individual	total	individual	total
Peniophora gigantea	828	+6	+4968	0	0
Leptographium lundbergii	622	+5	+3110	-1	- 622
Discula pinicola	246	-2	- 492	-5	-1230
Ceratocystis pilifera	221	-2	- 442	-3	- 663
Basidiomycetes sp.1	199	0	0	-6	-1194
Sclerophoma pityophila	151	-1	- 151	-4	- 604
Stilbella subinconspicua	144	-1	- 144	-5	- 720
Dendrophoma eumorpha	143	+4	+ 572	-4	- 572
Trichoderma lignorum	128	+8	+1024	+8	+1024
Basidiomycetes sp.2	111	0	0	-6	- 666
Not sporulating	108	+4	+ 432	+2	+ 216
Cephalosporium acremonium	83	-4	- 332	-6	- 498
Hirschioporus abietinus	81	-3	- 243	-7	- 567
Leptographium serpens	79	-2	- 158	-5	- 395
Botrytis cinerea	72	+6	+ 432	0	0
Summary (general) biotic effect			**+8576**		**-6491**

The biotic series method, applied here, was presented yet earlier (Mańka 1970). According to it the plus values in tables 3 and 4 are indicating an effect limiting the growth of the organism tested. The summary or general biotic effect should be considered as expressing the effect of the whole fungal community, which in turn represents in any way the environment whichfrom it was isolated, on the organism inwestigated.

Table 4 - The effect of the community of stump fungi from a 30 years old pine stand (Forest Laski, section 17 d) on Fomes annosus and on Peniophora gigantea

Species of fungus	Frequency of occurence	Fomes annosus		Peniophora gigantea	
		biotic effect		biotic effect	
		individual	total	individual	total
Peniophora gigantea	629	+4	+2516	0	0
Fomes annosus	358	0	0	-4	-1432
Basidiomycetes sp.	341	-2	- 682	-4	-1364
Ceratocystis minor	317	-2	- 634	+3	+ 951
Leptographium lundbergii	165	+2	+ 330	0	0
Sclerophoma pityophila	145	-1	- 145	-4	- 580
Cephalosporium acremonium	105	-4	- 420	-6	- 630
Trichoderma lignorum	99	+8	+ 792	+8	+ 792
Not sporulating	76	+1	+ 76	0	0
Mortierella isabellina	61	-1	- 61	-3	- 183
Paecilomyces elegans	61	-3	- 183	-3	- 183
Penicillium spinulosum	53	+2	+ 106	-3	- 159
Discula brunneo-tingens	50	-1	- 50	-1	- 50
Aposphaeria fuscomaculans	44	-5	- 220	-4	- 176
Ceratocystis piceae	36	-3	- 108	-5	- 180
Summary (general) biotic effect			+1317		-3194

The tables 3 and 4 confirm once more, and elucidate to some extent, the fact presented formerly, that pine stands in the Forest Zielonka are less dangered from Fomes annosus than those in the Forest Laski. The effect of fungal communities isolated from stumps of Zielonka, on Fomes annosus, turned out to be more limiting (+8576) than the effect of the community of stump fungi from Klęka (+1317), and in the case of Peniophora gigantea more favouring when from Zielonka (-6491) if compared with the effect of the community from Klęka (-3194). The investigations concer - ning other pairs of pine stands gave similar results (with one exception) though not always of a so high evidence.

Data given in chapters I to III deal with pine stumps and with fungi colonising them, from the point of view of the Fomes annosus problem problem. Rishbeth (1950, and a series of subsequent papers) sharply pointed out the im - portance of stumps for this problem. It is now clear, that the stump phenomenon brings into focus the majority of the Fomes annosus aspects, including the environmental aspect. The latter seems to be far from scientific exploration, probably because of the lack of proper methodical founda - tions. May be, this paper brings some new possibilities in this respect. In chapters I and III is considered mainly the effect of the external environment. Communities of fungi seem to be a good criterion to get a quantitatively expressible idea on the effect of a given environment on an organism, here on Fomes annosus and on Peniophora gi - gantea. Chapter II refers, to some extent, to the effect of the internal stump environment (breadth of annual rings) on Fomes annosus, a problem touched also by Cour - tois (1970), and more indirect by other authors.

The paper presents also some results more directly connected with the problem of butt rot control (possi - bility of escaping from Fomes annosus infection because of existing of a stump-infection free period, diminishing the threat from infection by taking off discs from stumps exposed some months, etc.), however requiring to be veri - fied in praxis.

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DISCUSSION OF

INFECTION PHENOMENA

F. Roll-Hansen (Norway), Chairman, M.D. Srago (USA), D.L. Lane (USA), Hertha von Aufsess (Germany)

INTRODUCTION

Srago presented the paper: The influence of soil biological factors on the mycelial growth of Fomes annosus. By M.D. Srago and F.W. Cobb.

DISCUSSION

Stambaugh: How did you determine that the strands were formed by Fomes annosus?

Srago: I brought the fungus back on agar media.

Myren: I remember a paper on effect of soil moisture content on growth of wood decay fungi. Are you aware of this paper?

Srago: In sterile soil held at field capacity I found no growth of F. annosus.

Dimitri: Do you think F. annosus forms any mycelium strands in the soil under natural conditions that would produce infection of the roots of standing trees?

Srago: Emotionally yes, but I have never observed it.

Yde-Andersen: Have you considered that by pasteurizing the soil you will get released more nutritions. You will, for example, get more nitrogen, more sugar, etc., so that the higher growth rate of Fomes may be an effect of the pasteurization?

Srago: I hadn't considered nutrition at all.

Myren: Did you do any work with germination of spores?

Srago: No.

Myren: Did you notice development of any resting structures like a chlamydospore?

Srago: No.

Courtois: In your regression model, to what extent did other fungi explain your regression?

Srago: It was not a model but an equation. Factors used were other fungi, bacteria, and actinomycetes associated with it.

Courtois: In our experiments we have found a synergistic relationship between F. annosus and Armillaria mellea. Thus, for example, the intensity of wood decomposition by F. annosus was not checked if the spruce wood was occupied by A. mellea before. In what extent did you consider in your experiments the effect of soil nutrients, especially the available nitrogen, carbon sources, minerals, and trace elements, and the effect of the acidity?

Srago: I tried different carbon sources added to the wood blocks without any effect. I didn't seriously look at this aspect.

Rishbeth: I have occasionally seen sporophores in the litter, as many other investigators have, or at the soil surface. In only one occasion I was reasonably sure that the sporophores arose from a mycelial strand rather than from a root; this was on heavy clay at a very moist time and in a situation where it was rather few conifers, in fact Norway spruce, but a large proportion of broadleaf trees. In this clay the mycelial strand, which was about 2 mm wide, rose vertically through the soil, and the sporophore was formed on the soil surface. I think that the same situation appears with Polyporus schweinitzii.

INTRODUCTION

Lane presented the paper: Studies on Fomes annosus and the infection of pine seedlings. By C.L. Lane and W. Witcher.

DISCUSSION

Dimitri: I have had no problem getting infection of pines and spruces under sterile conditions (100% infection). This was not comparable to natural conditions, however. Under nonsterile conditions we never got more than 1% infection. In other words our results were the same as yours.

Myren: The area of contact between inoculum and seedling was relatively small. Have you tried notching the seedling?

Witcher: Yes, it was notched. Also the seedling pictured was infected and had mycelium growing on it, but it did not die. Some of the seedlings had tiny fruit bodies on the side.

Myren: You wounded the seedling?

Witcher: Yes.

Myren: Was the inoculum block also cut to fit to the seedling?

Witcher: Yes, many of the blocks had bark on, and we cut through the bark to the wood of the inoculum block.

Rishbeth: Was it conidia or basidiocarps, fruiting on the seedlings?

Witcher: They looked like the beginning of tiny basidiocarps. They weren't actually conks.

Myren: Did you try placing the inoculum block in contact with the roots rather than in contact with the stem as you showed us?

Lane: We did put some in the soil. In a plantation seedlings planted over F. annosus-inoculum were stimulated.

Klingström: Are there methods available to make field inoculations with smaller seedlings?

Lane: We haven't been able to inoculate seedlings in the field.

Dimitri: We were always able to infect seedlings or one-year-old trees. We got 80% successful infection after four or six weeks. But after half a year Fomes was not found more.

There must be an active resistance-reaction in these seedlings. It is a biochemical problem.

Lane: We need a method of making a tree sick that doesn't kill it.

Dimitri: Explained his method of seedling inoculation.

Sylvestre: What was the age of your seedlings?

Dimitri: Four weeks. We tried to assess the different resistance of clones in growth chamber, that is completely the same environment. The same strain of F. annosus with different clones of the host. And one clone with different strains of the fungus.

Pratt: Gibbs set up an experiment to evaluate susceptibility of different species. He dug trenches 30 yards long, 18 inches deep, and 2 feet wide. He filled the trenches full of decayed root material (Scotch pine) and planted 10 species in 6×14 replicated plots. The idea was to provide equal opportunity for infection. After three years very few plots with killing so far. Some indication Douglas fir is going out more so than others.

Myren: Erik Jorgensen did similar thing in Ontario and did get some killing.

INTRODUCTION

Von Aufsess presented the paper: Microscopic appearance of wood decomposed by Fomes annosus.

DISCUSSION

Kuhlman: Were those naturally infected roots?

Aufsess: No, they were infected in the laboratory. But we found the same pictures in naturally infected wood.

Courtois: In one of your pictures there were holes inside the cell walls; the holes were similar to soft rot holes; did you reisolate the fungus from this area? In one of the last pictures we saw cells with contents which might indicate attack by bacteria; did you reisolate the fungus from this area?

Aufsess: We did not reisolate the fungus, but the changes were examples of wood-rotting fungi and the enzymatic action they have. The inclusions in the cells are only found when F. annosus has affected the wood, with other fungi or bacteria we did not find them.

Pratt: Did you find in the early stages of decay, more cell wall breakdown in spring wood than in summer wood?

Aufsess: Generally we found more in summer wood than in spring wood.

Cobb: Any explanation for this difference?

Aufsess: No.

Cobb: We have observed the same thing in fir in the West where we got a laminated rot because the fungus takes the summer wood before it takes the spring wood.

Shain: Did you find F. annosus in the reaction zone in spruce?

Aufsess: Yes, we found F. annosus-mycelium.

Shain: Is the reaction zone a product of the fungus or of the tree?

Aufsess: We found the reaction zone without the fungus suggesting the reaction zone may be a product of the tree. The fungus must be in the area though.

Johansson: Infection of spruce through wounds made by tractors has been investigated in Sweden by Hyppel. Johansson reported on the research work carried out and the results.

Yde-Andersen: 60-year-old Norway spruce stands in Denmark had been clearcut. The stumps were examined and wounds within 1 meter of the stump were recorded. It was not found more Fomes in wounded than in non-wounded trees.

Wallis: What size wound? With western hemlock this was very important. Small wounds had no infection whereas infection occurred with large wounds.

Yde-Andersen: In Denmark our wounds are not so big as those in the USA.

Ross: Asked Lundeberg to compare Hyppel's data with Lundeberg's data presented at Minneapolis. Lundeberg's data had little indication of A. mellea or F. annosus. Does this mean A. mellea and F. annosus are able to infect the roots much later after wounding?

Lundeberg: I don't think so, I just took cores and Hyppel excavated the entire root and took it to the lab. Perhaps this accounted for the difference.

Dimitri: Reported results similar to Shigo's with early invasion of wounds by bacteria and fungi imperfecti. Only later were wood rotters found.

Wallis: Fomes is common in scars on western hemlock. Nine to fifteen years later up to 10 meters up stem. Stereum produced less damage.

Russell: On western hemlock scars near the ground and underground scars are the critical ones for entrance of F. annosus.

THE INFLUENCE OF ENVIRONMENT UPON THE PHYSIOLOGY OF PENIOPHORA GIGANTEA ON PINUS TAEDA

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INTRODUCTION

The competitive saprophytic ability of Peniophora gigantea Massee for dominance in pine stump colonization, as noted by Rishbeth (5), provides a potential means for the biological control of Fomes annosus (Fr.) Karst. Recently, suppression of the pathogen in fresh stumps of Pinus taeda L. has been attempted by increasing basidiocarp production of P. gigantea to coincide with the time of thinning (1). The efficacy of this procedure is contingent upon, amongst other factors, the influence of environment upon growth, sporulation, and spore germination of the fungus. These factors constitute the objectives of the present work as conducted on P. taeda in the Piedmont of North Carolina.

MATERIALS AND METHODS

Seasonal Inoculations

To determine the influence of season and site exposure upon growth and incubation time to fruiting, inoculations of P. taeda stem sections (90 x 8-14 cm), sealed at one end, were made with water suspensions of 10-day-old oidial cultures of P. gigantea during December, April, July, and October of 1968-69. The sections, in replicates of 8-16, were placed on the forest floor in each of three adjacent P. taeda sites selected for density of crown canopy, namely 1) closed, 2) partial, and 3) none (clear-cut area). At intervals of 4-5 weeks following the inoculations, two sections from each site were collected to determine growth of the fungus by Alizarin test (3) and verification by tissue isolations on a selective medium (4). To obtain supplemental data on fruiting under the closed canopy only, additional seasonal inoculations were made, namely 1) pine stem sections (50 x 8-14 cm) piled in several replicates of 12 and covered with pine branches, 2) stumps in replicates of 5-12, and 3) 90-cm stem sections at opposite ends with F. annosus and P. gigantea in replicates of 8-12. Bi-weekly inspections were made until basidiocarp production was complete on all sites.

Spore Physiology

Basidiospore release

Hymenial sections (3 x 3 cm), with bark substrate included, were cut from a single fresh basidiocarp of P. gigantea and exposed to constant temperatures of 5, 15, and 30C and relative humidities of 32.5-34.5 and ∿100 percent in three replicates of all combinations. Spores released from 1 cm^2 of each section were collected in vials at intervals of 24 hours through 4 days. The spore deposits were suspended in water and counted by means of a haemocytometer.

Spore germination

Basidiospores were collected from fresh basidiocarps as needed; oidia were obtained from washings of 2-week-old malt agar cultures. Germination of both spore types in aqueous suspension was tested at 23C on the following substrates: 1) distilled water, still and aerated; 2) 1.5% malt extract, still and aerated; 3) 1.5% malt extract agar and the same amended with a cold water extract of fresh P. taeda stump wood; and 4) P. taeda wood from the inner and outer rings of a

freshly cut stump. After optimum incubation, spores on substrates 1-3 were enumerated microscopically while those on substrate 4 were removed by washing, concentrated on 1.2μ pore filters, and treated with immersion oil and analine blue for direct visualization. Dry deposits of both spore types were also tested on fresh P. taeda stump wood and evaluated by the method used with substrate 4.

Temperature limits for germination were determined by spraying fresh stump-wood sections (2 x 2 x 0.5 cm) with separate aqueous suspensions of basidiospores and oidia. The sections, in closed petri dishes, were exposed for 1, 3, and 6 hours to a temperature range of -5 to 45C at 5-degree increments. Following exposure, the dishes were incubated at 30C day, 20C night, 12-hour photoperiod, and 90 percent relative humidity for 1 week. The experiment replicated three times, was then evaluated on the presence or absence of mycelial growth of P. gigantea emanating from the wood sections.

Inoculum concentration in relation to colonization efficiency of the two spore types was tested by inoculation of P. taeda stem sections (45 x 8 cm) with aqueous suspensions of 200,000, 100,000, 2,000, 200, and 20 spores per section. The inoculated sections, covered to prevent desiccation, were incubated at 30C with a 12-hour photoperiod and 40-50 percent relative humidity until sampled after 1 and 3 weeks. The experiment, replicated twice by spore concentration and incubation period, was evaluated by growth determination as measured by Alizarin test (3) and tissue isolation on a selective medium (4).

RESULTS

Seasonal Inoculations

Differences in growth of P. gigantea in 90-cm end-sealed P. taeda stem sections as influenced by season and site are summarized in Table 1. The temperature effect is apparent in growth maxima following the spring (3.2-3.5 cm/week) and summer (3.6-5.5 cm/week) inoculations as compared to minimal growth (1.6-2.6 cm/week) obtained from fall and winter inoculations. The modifying effect of site differences was most pronounced in the summer and winter inoculations with the growth optima occurring under a closed canopy and no canopy, respectively.

In the closed canopy site, incubation time to fruiting of P. gigantea ranged from 7-29 weeks in 90-cm end-sealed stem sections, 5-26 weeks in piled and covered 50-cm stem sections, 9-30 weeks in 90-cm stem sections with opposing growth of F. annosus, and 25-33 weeks in stumps (Figure 1). Again the temperature effect was

Table 1.--Average growth (cm/wk) of P. gigantea in P. taeda stem sections as influenced by seasons of inoculation and site exposure.

Season of Inoculation	Incubation Period (wks)	Crown-density of incubation site		
		Closed	Partial	None
Winter (Dec.)	26	1.6	1.9	2.6
Spring (Apr.)	27	3.5	3.4	3.2
Summer (Jul.)	9-15	5.5	4.7	3.6
Fall (Oct.)	16	1.7	1.5	1.8

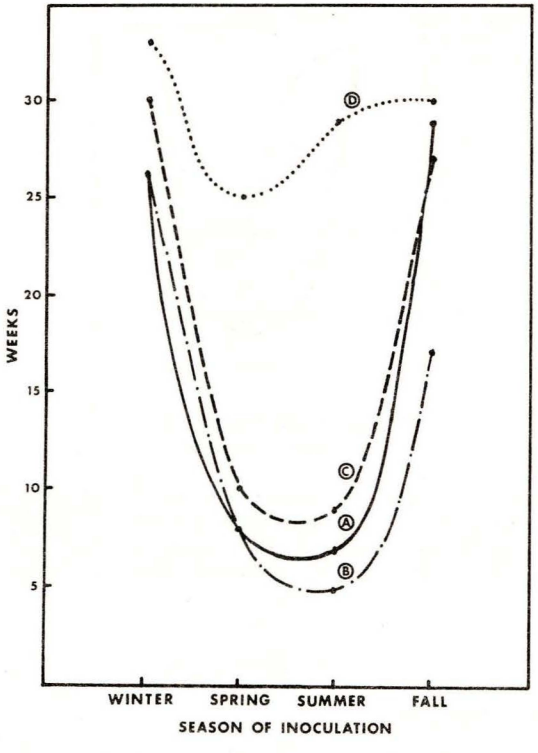

Figure 1.--Time to fruition of P. gigantea in seasonally inoculated P. taeda stem sections (A) end sealed, (B) piled and covered, (C) with F. annosus and (D) stumps, all under a closed canopy site.

quite evident with fruition time minimal following spring and summer inoculations. Piling and covering the smaller stem sections hastened fruiting in only the summer and fall inoculations. Inhibition of P. gigantea by F. annosus is suggested by the slight increases in fruition time over that of P. gigantea alone.

Site exposure effects upon time to fruition in the larger stem sections are compared in Figure 2. Incubation periods were consistently temperature related on all three sites with the shortest periods occurring under canopy protection. At fruiting level, minimum air temperatures were nearly the same on all sites, however, maximum air temperatures varied from 30C under the canopied sites to 43C on the open site.

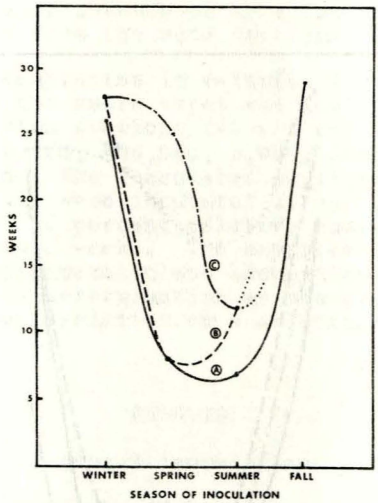

Figure 2.--Time to fruition of P. gigantea in seasonally inoculated 90-cm P. taeda stem sections under crown canopy conditions of (A) closed, (B) partial and (C) none.

Spore Physiology

Basidiospore release

The interaction of three temperatures and two levels of air moisture upon basidiospore release of P. gigantea is shown in Figure 3. Maximum spore production occurred at 15C under high humidity. Low humidity sharply curtailed sporulation and release at all temperatures, however, basidiocarps stored under

this regime at 5 and 15C resumed sporulation when transferred to the high humidity regime.

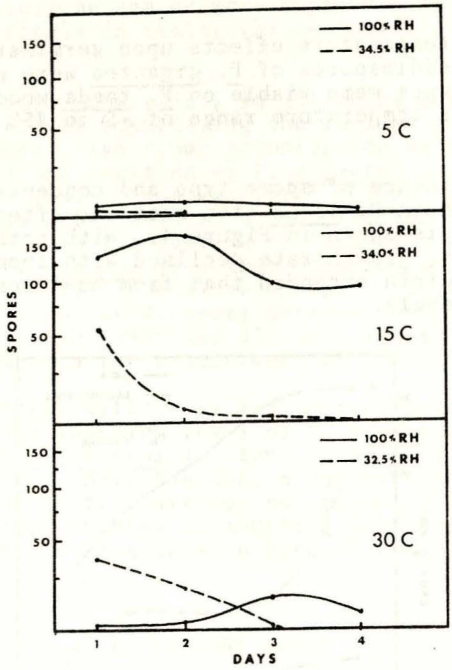

Figure 3.--The influence of air moisture and temperature upon basidiospore release (x50,000) from 1-cm^2 of P. gigantea hymenium.

Spore germination

At a temperature optimum of 23C, basidiospore germination of P. gigantea, unlike that of oidia, was nutrient dependent. Germination was nil in distilled water, regardless of aeration, whereas oidia germinated well under both still and aerated treatment (74 and 79 percent). In contrast, germination of basidiospores in aerated and still malt liquid was 69 and 76 percent respectively but less than that on malt agar (97 percent) or malt agar amended with P. taeda wood extract (99 percent). Spores applied directly to inner and outer rings of fresh P. taeda stump wood yielded germination levels of 95 and 94 percent respectively for basidiospores and 79 and 83 percent respectively for oidia. When basidiospores were applied dry to fresh stump wood, only 53 percent germinated in contrast to 95 percent germination when

comparable spores were atomized in water suspension. Likewise, oidial germination was lower (40 percent) in the dry state and higher (71 percent) when free water was present.

Lethal temperature effects upon germination of oidia and basidiospores of P. gigantea were not found. Both spore types were viable on P. taeda wood after exposure to a temperature range of -5 to 45C for 6 hours.

The influence of spore type and concentration upon linear growth in P. taeda stem sections after 1 and 3 weeks at 30C is shown in Figure 4. With both oidia and basidiospores, growth rate declined with inoculum dosage. Growth from oidia exceeded that from basidiospores at all dosage levels.

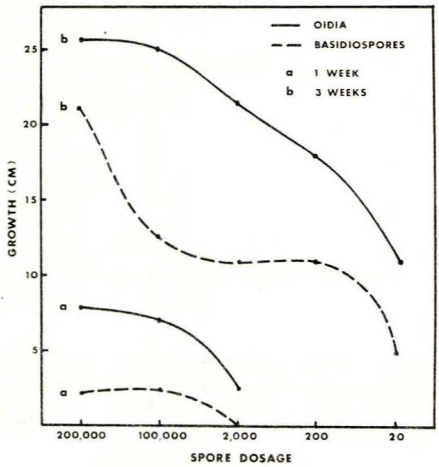

Figure 4.--Influence of spore type and dosage upon linear growth rate of P. gigantea in P. taeda stem sections at 30C.

DISCUSSION

As expected, the growth rates and fruition times of P. gigantea in excised stem wood and stumps of P. taeda are largely a function of temperature (Table 1, Fig. 1). That such seasonal effects can be modified to some extent by conditions of site exposure was demonstrated (Fig. 2). Within the unresolved limits of yearly variations and climatic transition, our data provide a schedule and techniques for synchronizing the fruition of P. gigantea with thinning operations. Fortunately,

the relatively closed canopy of first-thinning stands favors development of the fungus during optimal spring and summer conditions (Table 1, Fig. 2). Thinning during other seasons and on more exposed sites should be planned accordingly in timing the manipulation of P. gigantea.

The greatest uncertainty in attaching any reliance upon this method of suppressing the incidence of F. annosus in competitive stump colonization is the extreme sensitivity of the fruiting of P. gigantea to drying conditions. This restriction was first emphasized by Rishbeth (5) and is substantiated by our limited laboratory tests (Fig. 3). In the context of "climatic escape", i.e. avoidance of F. annosus by summer thinning as advocated by Driver and Ginns (2), the differential in lethal temperature effects upon basidiospore germination of P. gigantea and F. annosus, i.e. >45C and 45C (6), respectively, would favor the former in the enhancement of preventive control

The practical efficiency and control gains of manipulating the inoculum level of P. gigantea for dominance over F. annosus in stump colonization remains open to question. Nevertheless, a better understanding of this natural control system, as attempted here, merits further attention in view of mounting restrictions upon the use of various chemicals in disease control.

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ВЛИЯНИЕ РУБОК УХОДА НА ПОРАЖАЕМОСТЬ СОСНОВЫХ НАСАЖДЕНИЙ КОРНЕВОЙ ГУБКОЙ

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ВВЕДЕНИЕ

До сих пор дискуссионным является вопрос о путях проникновения и распространения гриба Fomitopsis annosa в лесных насаждениях. Одни исследователи считают, что основным путем заражения здоровых насаждений являются пни свежесрубленных деревьев. В Англии и некоторых других странах существует мнение, что корневая губка приходит в насаждения вслед за первыми рубками ухода (I, 2, 3). Другие исследователи (4, 5, 6) не придают большого значения пням в распространении инфекции и считают корневую губку обычным обитателем лесной подстилки, из которой при определенных условиях она переходит в корни. Одной из причин отсутствия единого взгляда по данному вопросу является недостаточная изученность влияния рубок ухода на поражаемость насаждений корневой гнилью.

МЕТОДИКА

Исследования по влиянию рубок ухода на поражаемость

насаждений корневой губкой проводились в сосняках Белорусской ССР. Было подобрано 6 участков сосновых насаждений площадью до 1000 га каждый, в которых наблюдалось развитие корневой губки. Эти участки были представлены чистыми сосновыми насаждениями I-II классов возраста, в которых рубки ухода были проведены не на всей площади. Часть их осталась не тронутой рубками ухода. Для оценки влияния рубок ухода на распространение и развитие корневой губки было проведено специальное фитопатологическое обследование насаждений во всех участках и заложены пробные площади в сосняках, где рубки ухода проводились или не проводились.

При обследовании устанавливалось состояние насаждений, степень их пораженности корневой гнилью, количество прогалин на выделе, средний диаметр прогалин и характер усыхания деревьев. Данные группировались по возрасту и типам леса в зависимости от того проводились или не проводились рубки ухода, их интенсивности и времени проведения.

РЕЗУЛЬТАТЫ И ОБСУЖДЕНИЕ

Исследования показали, что корневая губка встречается как в насаждениях, в которых рубки ухода проводились, так и в насаждениях, в которых они не были проведены. При этом различия в степени поражения между этими категориями насаждений в возрасте 15-20 лет незначительны. Это объясняется тем, что первые рубки ухода в исследуемых сосняках начали проводится с 14-15-летнего возраста и возможное влияние их может сказаться только через какой-то более длительный промежуток времени. Более значительное влияние рубок ухода на пораженность их корневой губкой проявляется в сосновых насаждениях 25-30-летнего возраста. В этом возрасте в отдельных участках встречаемость очагов корневой губки в сосняках, пройденных рубками ухода, в 2-3 раза больше, чем в насаждениях, не тронутых рубками ухода. Однако, и в насаждениях не пройденных рубками не менее 25% выделов было поражено заболеванием.

В сосновых насаждениях I-II классов возраста, не тронутых рубками ухода, площадь, занятая очагами корневой гнили, составила 3,9 - 5,6 % от общей площади обследованных участков, в то время как в сосняках, в которых были проведены 6-8 лет назад рубки слабой или средней степени изреживания, она равнялась от 6,8 до 7,9 %. Это происходит за счет того, что в результате рубок возникают дополнительные очаги поражения, приводящие к увеличению количества больных деревьев и большой расстроенности насаждений. Пни свежесрубленных деревьев во многих случаях не являются центрами дополнительно образующихся очагов усыхания. Это дало основание заключить, что распространение корневой инфекции в сосновых насаждениях происходит не только через пни свежесрубленных деревьев, а также через

обнаженные и поврежденные корни ослабленных и усохших деревьев и лесную подстилку.

Таблица I.— Влияние рубок ухода на степень поражения насаждений корневой губкой

Название лесничества №№ участков	Вид мероприятия	Площадь очагов корневой губки, га	Степень поражения насаждений гнилью, %
Леснянское I - 3	Рубки ухода проводились	213,9	7,3
	Рубки ухода не проводились	309,3	3,9
Полонковское	Рубки ухода проводились	27,7	6,8
	Рубки ухода не проводились	294,0	4,0
Амговичское 5 - 6	Рубки ухода проводились	487,2	7,9
	Рубки ухода не проводились	460,8	5,6

Неодинаково влияние рубок ухода на развитие и распространение корневой гнили в сосновых насаждениях разных условий местопроизрастания (типов леса). Отмечено, что в условиях сосняков вересковых изреживание насаждений при рубках ухода в меньшей мере способствует появлению новых очагов усыхания, чем в сосняках мшистых. Это обусловлено различиями в распределении корневых систем сосны в разных условиях произрастания. В мшистом типе леса сосна развивает поверхностно-стелющуюся корневую систему с выходом большого количества корней в подстилку под моховым покровом, где они могут использовать влагу, выпадающую в весенне-летний период и большое количество питательных веществ, освобождающихся при разложении растительных остатков.

При проведении рубок ухода в этих условиях в результате трелевки спиленных деревьев моховой покров местами нарушается, что приводит к обнажению и повреждению корневой системы сосны, которая является благоприятным субстратом для развития гриба. Об этом свидетельствуют опыты, проведенные в Западной Германии (6), показавшие, что чем интенсивнее повреждения коры корней, тем более сильно и быстро развивается инфекция. В вересковом типе леса, вследствие того, что корни развиваются в минеральном грунте и практически не выходят на поверхность (в подстилку), при проведении рубок ухода происходит меньшее повреждение

корней, как это наблюдается в мшистом типе. Кроме того в сосняках вересковых вследствие сухости почвы складываются менее благоприятные микроклиматические условия для развития гриба. Поэтому вероятность заражения деревьев грибом и распространения инфекции после проведенных рубок ухода в данном типе леса меньшая, чем в мшистом типе.

На основании проведенных исследований сделаны следующие выводы:

I. Поражение сосновых насаждений корневой гнилью происходит как в насаждениях, пройденных рубками ухода, так и в насаждениях, в которых они не проводились. Проникновение грибной инфекции в здоровые насаждения может происходить через пни свежесрубленных деревьев и другими путями (лесная подстилка, поврежденные корни ослабленных и усохших деревьев и др.).

2. Проведение рубок ухода в сосновых насаждениях способствует появлению дополнительных очагов усыхания деревьев. Степень влияния рубок ухода на поражаемость насаждений корневой губкой неодинакова в различных лесорастительных условиях. Более сильное влияние наблюдается в сосняках мшистых по сравнению с сосняками вересковыми.

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INFLUENCE OF THE CLEANINGS ON KILLING OF PINE STANDS BY FOMITOPSIS ANNOSA

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Investigations have been made in pine stands of I-II classes of age in Byelorussia. We have determined the influence of cleanings on killing them by F. annosa. It has been found that the root-rot kills both the stands where cleanings were carried out and those without cleanings.

The infection can penetrate the stands through the stumps of felled trees and in other ways (injured roots of weakened and withered trees, forest floor). The effect of the cleanings on killing of pine stands is different in various growing conditions.

DISCUSSION OF CONTROL

C. H. Driver, Chairman

Myren: Presented unpublished results of stump treatment studies in Ontario, Canada. In these studies borax is doing the best job of control in red and jack pine. In 1971 stumps were treated with borax, sodium nitrite, or nothing (check) and were then inoculated with conidia. Two months later, stump colonization by Fomes annosus was for borax treatment, 1 of 36 red pine and 0 of 40 jack pine; for sodium nitrite treatment, 30 of 36 red pine and 19 of 40 jack pine; and for the check, 39 of 40 red pine and 40 of 40 jack pine.

Koenigs: Are the roots of borax-treated stumps remaining alive and therefore susceptible to direct root infection by spores?

Myren: No, some remain alive because of grafts but they are not infected.

Koenigs: Would you recommend borax?

Myren: Yes, in first thinnings or in second thinnings in areas with known minimal amounts of Fomes.

Russell: Presented results from paper entitled "Evaluation of chemicals to control infection of stumps by Fomes annosus in precommercially thinned western hemlock stands" by K. W. Russell, J. H. Thompson, J. L. Stewart and C. H. Driver, State of Washington, DNR report no. 33, 16 pp. 1973.

Hodges: Cutting stumps high in a precommercial thinning operation in eastern North Carolina allowed competitors of F. annosus to replace it before it reached the root collar.

Wallis: Leaving high stumps in precommercial thinnings has also been recommended for passive control in western Canada.

Krebill: Is chemical thinning still used in the western United States?

Russell: Because of instances of livestock death purportedly due to herbicides, we now use saws. We hope to return to herbicides.

Stambaugh: Presented paper entitled "The influence of environment upon the physiology of Peniophora gigantea on Pinus taeda."

Rishbeth: On the basis of Ross' extensive spore sampling, it would appear to be ill-advised to depend on natural inoculum of Peniophora.

Stambaugh: Yes, that is true. On the other hand, natural inoculum of Peniophora has negated many studies evaluating chemicals for stump protection.

Sylvestre: Presented control portion of paper entitled "Current research on Fomes annosus in France" mainly on Scytalidium as a biological control organism.

Laird: We also attempted biological control with Scytalidium on western hemlock. The fungus was unsuccessful in penetrating below 2 cm into the tissues of fresh stumps.

Klingstrom: Since Scytalidium was first discovered in Sweden, I was pleased to see you using it in France. I was confused that the fungus spread so slowly for you in living trees. In Sweden we have some huge spruces with natural decay that were inoculated with Scytalidium. Scytalidium has spread more than 1 meter in 2 years and Fomes can no longer be isolated from the wood.

Rishbeth: Can these contrasting results be due to inoculating living and dead wood?

Klingstrom: Yes, we inoculated into decayed heartwood.

I have been bothered by the term "immunizing commensal." The words are contradictory since immunizing means to make immune, whereas commensal means to grow in association with.

Rishbeth: I have discussed this contradition with Ricard.

Koenigs: Does the Scytalidium parasitize the Fomes?

Klingstrom: It produces an antibiotic substance that is effective in advance of the mycelium.

Driver: I propose the conference make statements with regard to control at least for a particular region of the world, starting with chemical control.

Hodges: I would recommend dry borax under southern forest conditions. Qualified to first thinnings or second thinnings where Fomes wasn't already a major problem.

Russell: Borax is effective in western hemlock providing stumps are cut level and is reasonably effective year round (being less effective in winter).

Myren: Borax is effective in Ontario for pine.

Russell: I'd add our pines in the West.

Srago: In California in precommercial thinnings of pines, no control is deemed necessary.

Parmeter: I'm not certain borax prevents residual tree infection under western United States conditions. We know stump infection is controlled.

Morrison: In British Columbia zinc chloride and ammate are effective for stump protection in Douglas fir and western hemlock. They are as effective as borax but don't have the winter breakdown experienced with borax in the West.

Greig: In England we use 20% urea on all species. Sodium nitrite is also used.

McAree: In Ireland we use 20% urea.

Hodges: Have you checked the effectiveness of urea in terms of mortality in the residual stand? Urea tends to mask Fomes by stimulating Trichoderma and after several years Fomes shows up although urea initially appeared effective.

Rishbeth: Agreed that Fomes does fruit on urea-treated stumps.

Redfern: We have been checking Sitka spruce following treatment with sodium nitrite. Found up to 10% Fomes in stumps that were treated by forest crews.

Hodges: In conversation with some foresters they report some failures with borax. This suggests that the chemical no matter how good can only be effective if properly applied.

_____: Question concerning accumulation of borax in soil.

Driver: Estimated 1-2 lb of borax applied per acre in the southern United States. This is comparable to the pound per acre of boron that is recommended for agricultural use every 3-5 years.

Hodges: My studies indicated 200-300 gm (0.66 lb) of borax per acre were applied on the stump.

Russell: In Washington we put on 3-4 lb per acre.

Rishbeth: The agricultural recommendation assumes uniform distribution, whereas stump treatment concentrates the borax in limited areas. At these places it might be at super optimal levels following stump decomposition.

Dimitri: Questioned the validity of stump treatment for control. Doubted that the stump surface is the main avenue of infection on the basis of German experience and the billions of spores Fomes produces.

General discussion followed on the stump surface vs. direct root infection as the primary infection court. Results in England, Scandinavia, and the southern United States supported the stump-surface theory. Dimitri, Skelly, and Stambaugh stated their reservations to this theory.

GENERAL SESSION

C. S. Hodges, Chairman

Hanlin: Discussed the details of conidial formation by F. annosus as determined by transmission electron microscope studies.

Hyphae arise from the mycelium and grow up to form conidiophores. These conidiophores swell at the apex to form a rounded ampulla. All over the surface of the ampulla small areas of the protoplast and associated wall bulge out synchronously. The ampulla wall consists of three layers; an outer electron transparent layer, a middle dense layer, and an inner transparent layer. As the wall bulges out the outermost wall layer breaks. The two inner layers then elongate to produce the slender denticle. The denticle swells at the apex to form the conidium, which is then delimited at its base by formation of a septum. Conidia consistently obtained two nuclei. The mycelium contains a typical basidiomycetous septum with a septal swelling and perforate septal cap.

Driver: Are the two nuclei in the conidia heterokaryons or monokaryons?

Hanlin: I don't know. On the basis of these studies, I couldn't draw any conclusions about the cytology.

Courtois: Did you ever find just one nucleus per conidium?

Hanlin: We only used one isolate in the study and all conidia had two nuclei.

Courtois: In Germany, working with different isolates, about 80% of the isolates had 1 nucleus per conidium, 10-20% had 2, and less than 5% had 3-5 nuclei per conidium.

Cobb: Did you ever observe the two nuclei migrating into the spore?

Hanlin: No. The whole process happens so quickly, I was unable to observe it.

Courtois: Did you determine the number of nuclei in the mycelium?

Hanlin: No. However, they usually occurred in pairs.

Froelich: Discussed the use of fertilization to prevent spread of F. annosus in established infection centers.

The study was established in 9 infection centers in an 18-year-old plantation of slash pine. All trees in each center were measured and classified as being healthy, infected, or dead. Each plot was 132 feet in diameter with a border strip 83 feet wide. The plots were stratified into three groups, high, medium, and low, on the basis of potential for further spread. One plot in each group was treated with 2000 pounds of sulfur, another with 2000 pounds of nitrogen fertilizer (16.5% NO_3, 16.5% NH_4), and the third served as the control. After treatment, the pH in the nitrogen plot fell to 4.5-5.0 and in the sulfur plot fell to 2.5-4.0 from an initial pH of 5.3-5.8. After three years pH in the sulfur plots was 4.3. Respiration was reduced one-fourth to one-half for 2 years after application of sulfur and slightly reduced for 1 year after application of nitrogen. After 2.5 years, the number of new infected trees was considerably less in the plots treated with sulfur. Treatment with nitrogen only slightly reduced the number newly infected trees. While sulfur is effective in reducing the spread of F. annosus in established infection centers, this treatment would only be practical on high-value sites.

Wallace: Were there any differences in the root systems among the treatments?

Froelich: In the nitrogen and sulfur plots there was considerable regeneration of lateral roots. It appeared that the F. annosus infection had been checked and new roots had proliferated. Some of these were up to 4 meters long.

Alexander: Would the drop in soil pH to 4.3 be enough to affect the interior of the root to where F. annosus would stop growing?

Froelich: I don't know.

Wallace: With Poria weirii the addition of fertilizer causes the tree to become greener. We used 2000 pounds of nitrogen and trees with crown symptoms turned green very quickly because of the proliferation of new roots. We didn't observe any effect of fertilization on the fungus itself.

Driver: In some cases with P. weirii I have observed that growth of the fungus in the roots stopped with no treatment, and with subsequent proliferation of new roots. Sometimes there is an increased growth rate in infected trees because of a 25% greater proliferation of roots over non-infected trees.

Roll-Hansen: Discussed some of the silvicultural methods used in Norway for the control of F. annosus and the danger of generalization.

Fomes annosus is widely distributed in Norway up to about the polar circle in the north. It occurs within a wide range of climatic and pedologic conditions and is known from more than 25 different host species. The silvicultural measures against the fungus will often vary with climate, soil, and host.

Near the ocean in western Norway there is an Atlantic climate with annual precipitation often between 1,000 and 3,000 mm. Here the conditions for vegetative growth of Picea abies are usually much better than for Pinus sylvestris; inland provenances of the pine are especially poorly adapted to this climate. In this Atlantic climate the pine may be at least as severely attacked by F. annosus as the spruce, particularly in the case of young stands, consisting of inland provenances. In sharp contrast to this P. sylvestris is seldom attacked by the fungus in eastern Norway where the climate is drier; the spruce, however, is equally susceptible in both western and eastern Norway. In Norway, the resistance of Larix spp. and Pseudotsuga menziesii seems to vary in response to the climate in much the same way as does the resistance of P. sylvestris.

Pinus sylvestris becomes increasingly resistant to F. annosus with increasing age of the trees, with butt rot being of little importance in this species. Picea abies, however, will suffer increased damage due to butt rot with increasing age.

On low sites pine has a higher wood production than spruce. On high sites, however, spruce produces far more than pine.

Within one tree genus there may be great variation between species as to resistance to F. annosus. Abies alba is the most resistant conifer used in Norwegian forestry; A. balsamea is among the most susceptible ones.

From the foregoing facts, we can see it would be dangerous to generalize with respect to silvicultural control practices against this fungus. For example, in western Norway it may be dangerous to plant P. sylvestris in a locality with high incidence of F. annosus and it may be dangerous to thin the stand without treatment of the stumps. However, in eastern Norway it is quite safe to use pine in high incidence localities, such as where clear-cutting of heavily attacked spruce stands has occurred.

Even in western Norway, pine trees more than 40 years old will seldom be damaged by the fungus; if a stand is not destroyed before that age it can be maintained, unthreatened by the fungus, whereas an infected spruce stand will be increasingly damaged by butt rot. Attack by the fungus requires shortened silvicultural rotation in spruce, but not in pine.

On high sites it may be economically right to plant spruce even where this species is prone to heavy attacks by F. annosus, spruce may still yield more than pine. On low, heavily infected sites the pine is a better species, especially in eastern Norway.

Where conditions fit A. alba, our best recommendation against annosus-attack is to use this tree species. But we cannot generalize and use all firs; A. balsamea would be a very bad choice.

Conclusion: Control measures against Fomes annosus cannot be generalized. They will vary with climatic and pedologic conditions and with the tree species.

Skelly: One of the most critical areas in the southeastern United States that needs further study is whether or not we have a growth loss due to F. annosus in addition to mortality. It is obvious that growth loss or yield occurs in other root diseases. There should already be much information in our records and it only remains to make a few additional measurements to determine if this is indeed true.

Yde-Andersen: We have tried to find if growth loss occurs in Norway spruce attacked by F. annosus, but considering the stand as a whole, we could not find any loss. In thinning experiments we can take away nearly 50% of the stand and still have the same increment. We can have about 50% of the trees with disease without affecting the increment of the stand as a whole.

Cobb: As I understand it, there is growth loss in individual trees but this is compensated for by additional growth in neighboring trees and there would be no growth loss in the stand as a whole. Increment would be put on other stems. That is perfectly reasonable as long as you have a more or less randomized distribution but where we have localized infection centers in the stand, this may not be true.

Froelich: If you had thinned a stand to its optimum spacing, death of a tree would not have an impact on the growth of the residual trees. In my studies on the influence of fire on F. annosus, I have taken detailed growth data on the trees remaining after thinning. These data show that many trees with conks have almost quit growing.

Skelly: Because a tree can make good growth even with a portion of the roots gone, we need to consider the degree of deterioration of the root system as well as disease incidence when we are trying to determine growth loss.

Hodges: Another subject worthy of consideration is the stabilization of infection. At least from the standpoint of mortality, many infection centers in pine stabilize after a certain period of time. This is well documented in the literature. However, I have never seen a satisfactory explanation of this phenomenom.

Driver: Does the opening up of the stand and increase in competition have anything to do with stabilization?

Hodges: That is a possibility. There are reports in the literature that trees on the margin of the stand appear to be more resistant to infection by F. annosus than trees on the interior of the stand. Essentially when the infection center gets large enough, the trees around the periphery of the center may react as the trees on the margin of the stand. Other workers have indicated that trees become more resistant with age but no substantiating data is available.

Parmeter: In California I have not seen any special evidence to indicate stabilization. We have large pockets of one-half acre or more and the disease is still spreading. Also age does not appear to be a factor because some of the stands are more than 100 years old.

Parmeter: What is the possibility of using mixtures of species in stands to reduce losses from F. annosus. In California spread and amount of loss in mixed stands appear to be related to the percentage of a single species in the stand. The closer you come to a well-mixed stand, the less likely you are to sustain damage. We think that it may have some possibilities as a silvicultural method of control.

Yde-Andersen: In Denmark on former arable and heath land we commonly have used a mixture of pine and spruce. After about 20 years, in most cases the pine has been cut and the result has been that at an age of 25 years, nearly 20% of the spruce are attacked. We have similar experience with mixtures of larch and spruce and pine and Sitka spruce. When pine was not removed, there was no attack on the spruce. F. annosus can spread from one species to another very easily. In mixed stands of Norway spruce and beech, there was nearly the same percentage of attack in the mixed stands as in the pure stands. In the mixed stands we were also getting heavy attack from Armillaria mellea as well. What little we were gaining on the one hand we were losing on the other.

Srago: Have you seen the fungus go from spruce to pine? We have not seen F. annosus spread from true firs to pine in California.

Yde-Andersen: We do in pine planted after spruce.

Dimitri: We get nearly the same result in Germany. If you have 80% spruce and 20% beech the result is nearly the same as 20% spruce and 80% beech. The question is an economical one. It may be better to have 20% butt rot in a pure spruce stand than 5% in a mixed stand. It is very difficult to get two species together that are completely compatible.

Roll-Hansen: In Norway we plant a mixture of Norway spruce and Abies alba on high, relatively warm sites. When we have to take out the spruce after a few years, we often get an excellent stand of Abies.

Sylvestre: Is it possible to use a mixture of certain species of hardwoods such as Robinia pseudoacacia or others which might produce antibiotic substances from decomposing leaves which would leach into the soil and inhibit the spread of F. annosus?

Krebill: There are several shrubs like Ceanothis and Alnus that fix nitrogen and which may change the soil microbiological characteristics to the extent that they may influence the spread of F. annosus.

Froelich: An understory of Lespedeza established by planting after a prescribed burn and which has been maintained for six years apparently has had little effect on the spread of F. annosus.

Shain: Commented that since forest fertilization is being used more and more and that this practice may influence the microbiological activity in the soil and indirectly F. annosus, some work should be started on the influence of fertilization on the spread of F. annosus.

Driver: Discussed the possibility of setting up a standard design for studies on stump treatment so that results would be comparable from area to area and from country to country.

Hodges: Indicated that such a design should allow for determination of the amount of disease in the residual stand at least 5 years after treatment as well as short-term evaluation in the stump 3-6 months after treatment.